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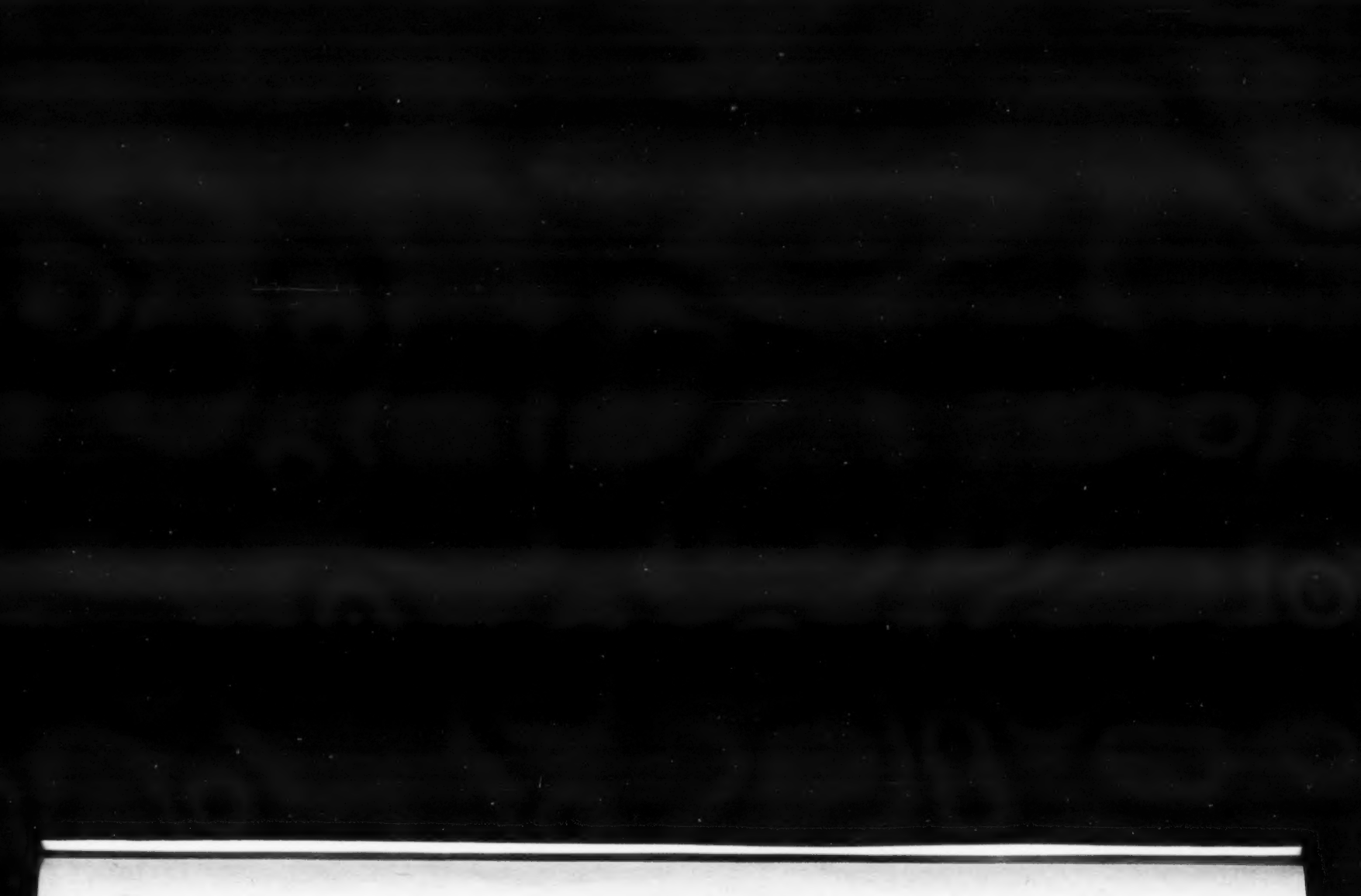

ON THE FORMATION OF THE TOXIC FLUID FOUND IN ISOLATED DUODENOJEJUNAL LOOPS[1]

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Although many authors have tried to explain the mechanism of death in high intestinal obstruction, no agreement on this subject has as yet been arrived at, and thus far no absolute proof has been advanced which would substantiate any one of the various theories put forward. (For exhaustive literature and critical surveys see 1, 2, 3, 4, 5.) The view most generally accepted at present is that death in this condition is due to the absorption of a toxic substance into the bloodstream, but the origin and nature of the poisonous element are by no means agreed upon. Most authors believe that the toxin is formed either in the lumen of the digestive tract, whence it may be absorbed by various paths, or in the intestinal mucosa itself, when part of it is supposed to be excreted into the lumen of the obstructed gut. In cases of intestinal obstruction the bowel always contains variable amounts of fluid, and experimental work has shown that, when the animals have died from high intestinal obstruction, this fluid has been found to possess very toxic properties (5). It seemed of interest to ascertain how long it takes for this fluid (1) to appear and (2) to become toxic.

A careful survey of the literature has not given information on these two points. Moreau (6) has studied the amount of secretion in isolated high intestinal loops of about 12 to 15 cm. in length, and has attempted to show that section of the nervous supply in the mesentery increases this secretion. When the nerves were not interfered with he did not find any fluid even after 18 hours. When the nerves were cut, he found varying accumulations of fluid—100 grams after 3 hours;

[1] Received for publication June 19, 1926.

[2] I am much indebted to Dr. Harvey B. Stone whose help and suggestions have facilitated very much the carrying out of this work.

in one animal 225 grams after 18 hours. In order to work out this problem systematically the following experiments were undertaken.

Healthy dogs, of about 9–12 kg. were chosen, and the technic was as follows: A loop of small intestine, about 15 cm. in length, comprising the third part of the duodenum beyond the pancreatic ducts, and the first part of the jejunum beyond Treitz's ligament, was isolated and both ends brought out of stab wounds through the abdominal wall. The parietal peritoneum and the subcutaneous fascia were sutured carefully around the stumps which were left open. The continuity of the intestinal tract was restored by an end-to-end anastomosis. Ten days were allowed for complete healing of the incisions and for the establishment of free drainage from both fistulas. The animals generally stood this procedure very well and remained in splendid condition for several weeks. About 10 days after this first operation both stumps were closed off by tying into them hourglass-shaped rubber stoppers with cotton tape or heavy silk. Some of the animals pulled out these stoppers, and to prevent this a well fitting linen jacket was employed. This device served not only to protect the stumps, but also enabled us to see if there had been any soiling produced by leakage.

We now had a typically isolated closed loop, with no defects in the mucosa and no suture lines. After a variable length of time the obstruction was released; any accumulated fluid was collected, its volume measured, filtered or centrifuged, and definite amounts injected intravenously, as soon as possible, into small dogs (4–5 kg.) in order to test its toxicity. The injected animals were observed carefully and if any of them died, an immediate autopsy was performed in order to determine the pathological conditions. The same animals with isolated draining duodenojejunal loops could be used several times, because the necrosed stumps healed very quickly. Sometimes the openings of the fistulas became very narrow, and instead of using the rubber stoppers it became necessary to dissect them out and tie them off with plain tape ligatures. When the time of closure lasted more than twenty-five hours, it was impossible to avoid sloughing and leakage. Under these circumstances it was found best to close both stumps with the Parker-Kerr suture under aseptic conditions. Since these dogs had to be sacrificed after one experiment, in their case

the making of Thiry-Vella fistulas was unnecessary, and isolated closed loops were made in the first sitting, combined with an end-to-end anastomosis.

The results and protocols of the experiments are given in Table I.

From a critical survey of our table several facts become clear. First of all, it is plain that, up to 36 hours, the loop content does not appear to be very toxic under the conditions presented. Only one of the control animals (No. 5) injected with the total loop content died, and even here the experiment cannot be considered as typical. Dog (VI-20) which yielded this material had an abscess in the abdominal wall communicating with the lumen of the occluded loop. The fluid itself was very thick and contained a quantity of pus, but it may have been concentrated very much by the absorption of water in the subcutaneous tissues. Moreover, the atypical findings at autopsy render it probable that the death of this control dog has to be attributed to some factor other than the toxic properties of obstructed loop fluid. Our results are constant enough to warrant the assumption that after 36 hours the fluid seems to become very definitely toxic. In the 50-hour experiment (XII), and in the 45-hour experiment (XIV), and in the 40-hour experiment (XI), the toxin must have been very powerful, especially in the first of these. Both controls died very quickly, one after 5 minutes, and the other after 15 minutes; the autopsies were done very carefully to exclude possible thrombosis or embolism in the big veins, in the pulmonary arteries, and in the right heart. Nothing was found except the typical pathological conditions in the duodenum which are known to belong to death from intoxication.

An analysis of the chart shows also that, even after a 10-hour obstruction, the accumulation of fluid is sufficient to produce a marked distention of the isolated loop (V-10), V-10. After 20 hours, the distention may be sufficient to cause perforation from gangrene (V-20). This may be an exceptional result, related to some idiosyncrasy in the venous system of this particular animal. There was surely no impairment of blood supply whatsoever as revealed by examination of the mesentery. But it is nevertheless a known fact, that some animals show a definite variation in the disposition of the veins of the last portion of the duodenum. This point would call for further investigation.

TABLE I

Results and protocols of the experiments
Obstructed animals

SERIAL NUMBER OF DOG AND CONDITION	TYPE OF CLOSURE	DURATION OF OBSTRUCTION	SYMPTOMS CONDITION OF STUMPS	LEAKAGE PRESSURE	AMOUNT COLLECTED	ASPECT OF FLUID	SUBSEQUENT COURSE PATHOLOGY
		hours			*cc.*		
III Good	Rubber stoppers	2	None Cyanotic	None Not increased	5	Slightly bloody Mucus	Normal 1 hour later stumps normal
V Good	Rubber stoppers	5	None Cyanotic	None Not increased	10	Slightly bloody Mucus	Normal 1 hour later stumps normal
V Good	Rubber stoppers	10	None Necrosed	None Increased	80	Thin, blood-stained Mucus	Stumps healed after sloughing. Very short, had to be corrected
VI Good	Rubber stoppers	10	None Necrosed	None Not increased, loop full	30	Thick, slightly bloody Mucus	Stumps healed very quickly
V Good	Rubber stoppers	15	Slightly sick at end of experiment Necrosed	None Very much increased	160	Thin, hardly blood-stained	Stumps heal up but remain much shorter
VII Good	Rubber stoppers	15	None Necrosed	None Not increased	20	Thick, dark-green grumous	Stumps heal up but remain much shorter
VI Good	Rubber stoppers (attempts to dilate caused perforation under skin)	20	None Necrosed	Slight Not increased, loop full	30	Thick, dark brown with pus	An abcess in the abdominal wall communicating with the loop was found. It healed up after evacuation by puncture in five days

V Good	Rubber stoppers	20	Looks sick, quickly worse, irresponsive, slight tremor in legs finally Necrosed	None Very much increased	100	Thin, blood containing	Dog died about fifteen minutes before the twenty hours had elapsed. Gangrene of loop and perforation. Definite pathology in duodenum No peritoneal reaction yet. No fluid
VI Good	Ligature after dissection	20	None Necrosed	Slight Very much increased	150	Thin, bloody	Stumps sloughed very quickly and healed
VII Good	Ligature after dissection	25	Sick after twenty hours, quiet, no vomiting Necrosed	None Definitely increased	130	Blood tinged	Dog sacrificed; definite hemorrhages in duodenum and jejunum. Beginning gangrene of loop. Distemper
VIII Good	Rubber stoppers	25	None Necrosed	Slight Slight	75	Blood tinged and thin	Stumps healed quickly, but dog died seven days later from unknown cause. Autopsy revealed nothing
VI Good	Dissection and closure by Parker-Kerr method	30	Dog quiet, looks sick Stumps perfect	None Increased	130	Hemorrhagic fluid thin	Sacrificed: moderate gangrene of loop, no perforation, no fluid in peritoneum. No definite pathology in duodenal mucosa
X Good	Closed loop and end to end anastomosis	36	Dog quiet, vomited once, later irresponsive Stumps perfect	None Increased	175	Hemorrhagic thin	Died after thirty-six hours. Anastomosis had ruptured. Loop gangrenous, not perforated. General peritonitis. 30 cc. Fluid in peritoneal cavity

TABLE I—*Continued*

SERIAL NUMBER OF DOG AND CONDITION	TYPE OF CLOSURE	DURATION OF OBSTRUCTION	SYMPTOMS CONDITION OF STUMPS	LEAKAGE PRESSURE	AMOUNT COLLECTED	ASPECT OF FLUID	SUBSEQUENT COURSE PATHOLOGY
		hours			*cc.*		
XI Good	Closed loop and end to end anastomosis	40	Dog sick, refuses nearly all food. No vomiting, suddenly worse Stumps healing	None Increased	110	Hemorrhagic and thin	Found dead after about forty hours. Gangrenous loop, perforation. Doubtful duodenal hemorrhages
XV Good	Closed loop and end to end anastomosis	43	Dog quiet, sick, refuses food, next day vomits once Stumps healing	None Increased	295	Thin hemorrhagic fluid	Sacrificed after forty-three hours. Very distended gangrenous loop. No peritonitis. Duodenal pathology indefinite. 2 to 3 cc. fluid in peritoneal cavity
XIII Good	Closed loop and end to end anastomosis	45	After twenty-four hours looks sick, takes no food at all Stumps healed	None Increased	70	Fluid thick hemorrhagic	Sacrificed after forty-five hours. Beginning distention of loop. Mucosa congested. No hemorrhages, no gangrene No duodenal pathology
XIV Good	Closed loop and end to end anastomosis	45	Dog refuses food. Not irresponsive and not very sick. Vomited abundantly after twenty-four hours. Stumps healed	None Increased	95	Darkened bloody fluid. Thick and foul	Sacrificed after forty-five hours. Definite distention. Loop mucosa congested and hemorrhagic. Beginning gangrene Duodenum congested
XII Good	Closed loop and end to end anastomosis	50	Sick but responsive vomited once Stumps perfectly closed	None Not increased		Thick, bloody fluid	Sacrificed after fifty hours. Loop not gangrenous and not distended. Mucosa very hemorrhagic. Slight hemorrhages in duodenum

TABLE I—*Continued*
Test animals

SERIAL NUMBER OF DOG AND CONDITION	SITE OF INJECTION	PREPARATION OF FLUID	AMOUNT INJECTED	SYMPTOMS	FURTHER COURSE AND PATHOLOGY	RESULT
			cc.			
1 Good	Vein of fore leg	Filtered on gauze and diluted up to 25 cc.	Total, 5	None	Normal	—
2 Good	Vein of fore leg	Filtered on gauze and diluted up to 25 cc.	Total, 8	None	Normal	—
3 Good	Vein of fore leg	Filtered on paper	25	None	Normal	—
3 Good	Vein of fore leg	Filtered on paper and diluted up to 25 cc.	Total, 15	None	Normal	—
3 Good	Vein of fore leg	Filtered on paper	25	Quiet for about one hour. Leg swollen at site of injection (abscess)	Two days later a small abcess broke down. Next day dog all right	—
4 Fair	Vein of fore leg	Filtered on gauze and diluted up to 25 cc.	Total, 15	Marked shock during the injection, only temporary (vomiting, defecating)	Normal	—
5 Good	Vein of fore leg	Filtered on gauze and diluted up to 25 cc.	Total, 12	Severe reation lasting for more than two hours (vomiting)	Next day (15 hours) found dead. Organs and peritoneum intensely congested. Very quick putrefaction (Gas bacillus)	Positive

TABLE I—*Concluded*

SERIAL NUMBER OF DOG AND CONDITION	SITE OF INJECTION	PREPARATION OF FLUID	AMOUNT INJECTED	SYMPTOMS	FURTHER COURSE AND PATHOLOGY	RESULT
			cc.			
3 Good	Jugular vein	Filtered on paper	25	Very quiet for two to three hours	Normal	—
6 Good	Jugular vein	Centrifuged	25	Marked shock during injection. Quiet during rest of day	Normal	—
3 Good	Jugular vein	Filtered on paper	25	Vomited twice in the hour following the injection	Normal	—
3 Good	Jugular vein	Centrifuged	25	Vomits during injection. Quiet, no more vomiting afterwards	Normal	—
6 Good	Femoral vein	Centrifuged	25	Vomits twice immediately after injection. Three hours later all right	Normal	—
7 Good	Jugular vein	Centrifuged	25	Vomits right after the injection	Still sick after twenty-four hours, all right after forty-eight hours	Positive
6 Good			25 (peritoneal fluid)	Vomits several times. Looks very sick	Remained sick for two days	

8 Good	Jugular vein	Centrifuged	25	Severe reaction, very sick quickly irresponsive	Dies after three hours. With typical duodenal pathology	Positive
9 Good			50	Severe reaction, very sick and quickly irresponsive	Dies after 3½ hours. Extreme duodenal pathology	
15 Good (4.5 kgm.)	Jugular vein	Centrifuged	25	No reaction. Later on very sick, paresis of hind legs. Responsive	Sacrificed after twenty-two hours. No pathology found.	—
16 Good (9 kgm.)			50	Marked reaction. No vomiting. Quickly worse and irresponsive	Dies after five hours with intense duodenal pathology	Positive
10 Good (7.5 kgm.)	Jugular vein	Centrifuged	25 25 (1 hour later)	No immediate reaction but soon very sick, dyspneic and irresponsive	Dies after nine hours with typical duodenal pathology	Positive
13 Good	Jugular vein	Centrifuged	25	No immediate reaction, later vomiting and defecating. After thirty minutes nearly moribund	Dies after four hours. Typical pathology	Positive
14 Good			45	Immediate severe reaction, fifteen minutes later moribund	Dies after three and one-half hours. With very extensive duodenal pathology	Positive
11 Good	Jugular vein	Centrifuged	15	Immediate shock extremely severe, fatal after five minutes	Beginning slight duodenal congestion. No thrombosis or embolism	Positive
12 Good			12 (diluted to 25)	Immediate shock, fatal after fifteen minutes	Definite duodenal pathology Thrombosis or embolism excluded	

Finally, other interesting findings were noted in some of the animals. The distention gangrene always started on the anti-mesenteric border, which is in complete accord with the findings of van Beuren (7). Some of our isolated loops showed this pathological condition in the very earliest stages.

Even when the gangrene is extensive in the distended loops, the fluid is not necessarily toxic, as evidenced by V-20, VII-25, and VI-30. And conversely, when the fluid is very toxic, we do not always find gangrene or distention (XIII-45). Hence the relation between the amount of necrosed tissued and the intoxication, which some authors have emphasized, is far from constant. In the case of Dog V-20, which died after 20 hours with a perforated loop, one may speculate on the cause of death. The loop fluid was not very toxic, but, nevertheless, definite pathological conditions (congestion, hemorrhage, and oedema) were found in the duodenum, which are generally accepted as among the typical findings in fatal cases of high intestinal obstruction. Although peritoneal shock cannot be excluded, it seems reasonable to admit that a massive peritoneal absorption of toxins was the determining factor. It would have been interesting to test the toxicity of the peritoneal content, but unfortunately this was not done.

What about the relation between the toxicity of the fluid and the survival of the obstructed animal? Three animals (XIII, XII and XIV), though yielding a powerful toxin, had no severe symptoms. Another one, however, (XI) in which the toxin was very powerful, died very quickly, although the autopsy revealed only slight pathological changes in the duodenum. There were a few hemorrhagic spots in the first two inches of the duodenum, just beyond the pylorus, and only a slight congestion of this whole region. But this dog (XI), in contrast to the others, had a tense distended loop, containing much more fluid, whereas one of the others had no distention at all (XII). Hence it is suggested that the death of the dogs depends on some other factor besides the presence of the toxic fluid. It is interesting in this connection to remember the recent experimental work of Stone and Firor (8) who emphasize the importance of pressure as a factor in the absorption of these toxins.

SUMMARY AND CONCLUSIONS

1. A series of experiments is presented dealing with the study of closed high intestinal loop fluid, and more especially, with the time required (1) for the accumulation of this fluid and (2) for the development of toxicity in it.

2. Sufficient evidence, it is thought, is given to prove that 10 hours is the minimal time required for the formation of appreciable amount of this fluid, and that this accumulation is able to cause distention gangrene as early as 20 hours after the obstruction.

3. Intravenous injection of 25 cc. of this fluid kills a small dog in a few hours, with typical symptoms and pathological findings assumed to constitute a criterion for its toxicity. It was found that the toxic properties, as a rule, take more than 36 hours to develop.

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STUDIES IN EXPERIMENTAL SYPHILIS

V. The Wassermann Test in Normal and Syphilitic Rabbits[1]

INTRODUCTION

JAROLD E. KEMP, ALAN M. CHESNEY and ALLAN K. POOLE

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The successful transmission of syphilis to rabbits in 1906 was naturally followed by attempts to study the behavior of the Wassermann reaction in these animals, both normal and syphilitic. Many reports were soon forthcoming but unfortunately the subject was not greatly clarified because the almost innumerable variations in technic did not permit of a ready comparison of the results of different investigators. As a result, considerable confusion has arisen in regard to this question, although the more recent work has on the whole shown a greater tendency toward unanimity. To give a detailed résumé of the contributions that have appeared on this subject is beyond the scope of this communication. Those interested should consult the recent summaries by Blum (1) and by Wakerlin and Carroll (2). It will not be amiss, however, to give here a brief outline of the situation as it appears to us at the present time after an examination of the literature.

HISTORICAL

Most of the earlier work on the Wassermann reaction in experimental syphilis of the rabbit was carried out in Germany and the general consensus of opinion arrived at was to the effect that the test could not be regarded as of value in this animal, since quite often normal rabbits were found that gave positive reactions; moreover, animals with outspoken syphilitic lesions not infrequently failed to give a positive reaction. Nichols (3), one of the earliest workers on the problem in this country, did not concur in this opinion. He found the

[1] Aided by a grant from the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.

test almost uniformly positive during the active stage of the disease and rarely, if ever, positive in normal rabbits.

The more recent observations have seemed to show definitely that with proper attention to certain technical details the test can be made to yield negative reactions with normal animals and positive reactions while the primary lesions are present. This has been the experience of Noguchi (4), Graham, Baldwin and Grigg (5), Heusinkveld and Carroll (6), Takenaka (7), and of Wakerlin and Carroll. The more recent contributions from German workers also indicate a tendency toward the view that the test can be successfully employed in experimental syphilis of the rabbit. In the experience of Sachs, Klopstock and Weil (8) a positive reaction in normal rabbits is a comparatively rare event. Blum, on the other hand, encountered a much higher percentage of positive reactions in normal rabbits than has been the experience of investigators in America or Japan. Whether this difference can be accounted for on the basis of variations in technic, inherent differences in the breed of the animals, or in the food supply in the different countries, is not clear as yet. Possibly all three factors are involved. Our own experience, as will be seen from what follows, tends to show that the test is a very useful adjunct in carrying out investigations in experimental syphilis of the rabbit, and is almost never found positive in normal rabbits.

TECHNIC OF THE TEST

The technic was essentially the same as that employed routinely in the Johns Hopkins Hospital and already described elsewhere (9). Only a brief summary of the method will be given here.

Serum. Blood obtained from the marginal ear-vein was allowed to clot at room temperature and the serum was allowed to stand over night in the ice-box. All tests were performed on the day following the bleeding and in no case was an interval of more than 24 hours permitted to elapse between the bleeding and the carrying out of the test. The serum was always inactivated by heating at 56°C. for 30 minutes. For the test, 0.1 cc. of whole serum diluted with an equal amount of normal salt solution was used, and 0.2 cc. was used as an anti-complementary control. (The total volume of reagents in the test amounted to 1.0 cc.). The dose of serum to be used, namely,

0.1 cc., was selected on the basis of a series of preliminary tests conducted with the serum of normal rabbits, wherein the preliminary incubation was carried out overnight at ice-box temperature. As the test was ultimately to be done with the preliminary incubation in the water-bath at 37°C., it was thought that we were within the limits of safety in selecting an amount of serum which never gave positive results with the ice-box method.

Antigen. Only one antigen was used throughout, namely, an alcoholic extract of beef heart fortified with 0.2 per cent cholesterol. It was so diluted that 0.2 cc., the amount used in the test, contained 8 to 10 antigenic units and was not more than one fourth the anticomplementary dose.

Complement. Pooled guinea-pig serum diluted 1–10 was used, the dose being 0.2 cc.

Hemolysin. Anti-sheep rabbit serum was used as the hemolysin, and titred daily against a fixed amount of complement, 0.2 cc. of a 1–10 dilution. Four units of hemolysin determined in this manner were used in the test and the dilution was so arranged that this amount was contained in 0.2 cc. It was found that when hemolysins with a titer of less than 1–2000 (minimal hemolytic dose) were used, there was a marked tendency to obtain anticomplementary reactions. For this reason the tests in the later work were always carried out with hemolysins of high titer, and under these conditions the anticomplementary controls always cleared promptly.

Corpuscles. Sheep corpuscles collected weekly and preserved in formalin and citrate solution were used in 5 per cent suspension in normal salt solution.

Incubation. First and second incubations were carried out at 37°C. for 30 minutes each. Hemolysin and cells were mixed together and allowed to stand in the ice-box for an hour or more before being added to the serum-complement-antigen mixture.

Readings. Readings were made at the end of the second incubation, and were reported on a numerical basis, as follows:

Complete fixation	4	positive
75 per cent fixation	3	suggestive positive
50 per cent fixation	2	doubtful
25 per cent fixation	1	suggestive negative
No fixation	0	negative

RESULTS

The results reported in this communication are based upon a total of 2680 tests performed upon approximately 900 different rabbits in the course of about 3 years. The animals were observed for varying periods, the maximum being 435 days. They were maintained upon a stock diet of cabbage, oats and hay, and the diet practically never varied.

Normal Rabbits. In 750 tests performed upon the sera of 600 animals prior to inoculation a positive reaction was encountered in 2 animals only, an incidence of 0.3 per cent. Both rabbits were adult albinos purchased from the same dealer at the same time, and the tests were performed within a few days after the animals arrived in the laboratory. Titration of the sera from these animals at the time they gave false positive reactions demonstrated that they contained an excess of anti-complementary substances over that found in normal rabbits and in syphilitic rabbits as well, tested at the same time. Two weeks later the tests were repeated and yielded a negative result in each case. Likewise, the titer of the anti-complementary substances was found to have diminished. Several subsequent tests were performed upon the sera of these animals, always with a negative result. They were later inoculated with syphilitic virus and in both the reaction became positive and behaved as in other syphilitic rabbits. As suggested also by Takenaka, the previous diet may have played a rôle in giving rise to the transient false positive reactions in these two animals.

Rabbits with indigenous infections. In the opinion of some of the earlier observers (10, 11, 12, 13) one of the main causes of positive reactions in normal rabbits is the presence of various infections to which the rabbit is susceptible. Fifty-two animals in this series were known to have severe snuffles, subcutaneous abscesses or coccidiosis at the time their sera were tested, but in none of them was a positive reaction obtained. The subsequent behavior of the reaction in these animals after they were infected with syphilis was in no wise different from that exhibited by other syphilitic rabbits. The serum from 2 animals infected with *Treponema cuniculi* likewise gave negative

results.[2] In this respect our results are in accord with those of other observers (14, 15, 16, 3).

Rabbits inoculated with non-syphilitic material. Eight rabbits inoculated with tuberculous material and 7 with *Streptococcus erysipelatis* were followed with repeated tests, and in no case was the reaction positive. Five animals injected with sheep cells for the production of hemolysin gave uniformly negative results.

Syphilitic rabbits. We are able to report upon the behavior of the Wassermann test in a series of 825 rabbits, most of which were infected with the Nichols strain of *T. pallidum*, some with the Truffi strain[3] and some with strains which we have isolated during the past four years. Altogether 9 strains have been tested. In general, it has not been possible to observe any differences in the behavior of the reaction that could be identified with any particular strain. Every strain with which we have worked has proved to be capable of evoking the reaction. On the whole, however, the reaction tended to remain positive longer in rabbits infected with the Nichols strain than in those infected with other strains, perhaps because of the more pronounced and longer tissue reaction shown by rabbits infected with the former. Where strains newly isolated were tested, the reaction became positive in the first animal passage, so that it was not necessary for the organisms to become well adapted to the rabbit in order that the animal might give a positive reaction. Moreover, no significant differences in the behavior of the reaction were observed after the freshly isolated strain had been passed through several generations of rabbits.

Most of the rabbits were inoculated by intratesticular injection, but observations were also made upon others inoculated by applying the virus emulsion to the exposed surface of a fresh or granulating wound in a manner previously described (16). In some of the animals the infection was permitted to unfold itself in its usual manner without any attempt to influence it in any way; in others, the natural course of the infection was altered by removal of the affected testis or by

[2] Dr. Louise Pearce of the Rockefeller Institute for Medical Research kindly supplied us with these sera.

[3] Professor W. Kolle of the Georg Speyer Haus, Frankfurt a/ Main, Germany, was kind enough to send us a rabbit inoculated with this strain of treponemes.

treatment with arsphenamine. In addition, observations were made upon rabbits first treated and then reinoculated. The results obtained under these varying conditions will be presented in the order mentioned.

BEHAVIOR OF THE WASSERMANN TEST IN UNALTERED SYPHILIS OF THE RABBIT

a. Testicular inoculation. A positive reaction in our experience does not appear, as a rule, until there is clinical evidence of the existence of orchitis, although it may be found as early as 2 days after the first clinical sign of infection in the testis. From the time of its appearance it increases rapidly in strength as the orchitis becomes more marked. Where the inoculum is scanty in treponemes, as when lymph nodes are used for inoculation, the incubation period of the disease is delayed and with it the appearance of the reaction. As a rule, the reaction remains completely positive with 0.1 cc. of serum as long as the initial lesion is present. In Figure 1 is shown a typical example of the behavior of the reaction with 0.1 cc. of serum from a syphilitic rabbit in which the infection was allowed to take its course without alteration. In this particular animal the reaction remained strongly positive for a period of about 50 days. The period of decline from a 4 plus reaction to a negative one was in this instance fairly short, about 20 days. In general the period of decline from 4 to 0 was longer than when the reaction changed from 0 to 4.

In Figure 2 is presented a composite curve obtained from 500 tests, upon a series of 75 animals, conducted at frequent intervals over a period of 435 days. Not all the animals were tested upon the same day but the tests were made at comparable intervals. In these animals the disease was allowed to take its course without molestation. The curve was obtained by adding the numerical values for the tests carried out on a given day of the disease and dividing the sum obtained by the number of tests performed. The resulting figure is then plotted as the ordinate. The slight elevation of the curve after the 100th day is explained by the inclusion, in the series, of 2 animals with a malignant type of the disease. These animals exhibited successive crops of generalised lesions and were responsible for the second rise in the curve about the 225th day of the disease.

Except for an interruption between the 175th and the 225th day of infection, their reaction was positive during almost the entire period of observation. It should be emphasized that they constitute exceptions to the rule.

Tests performed with dilutions of the serum in a series of rabbits indicated that the unknown reacting substance attained its greatest concentration from 2 to 6 weeks after the appearance of the orchitis, remained at or near this level for 2 to 3 weeks and then slowly diminished. The smallest amount of serum that gave complete fixation during this period of the disease was found to be 0.003 cc. In Figure 3 is shown the behavior of the reaction in an untreated rabbit when various amounts of serum were used in the test. In this particular animal the reaction reached its maximum about the 74th day of the

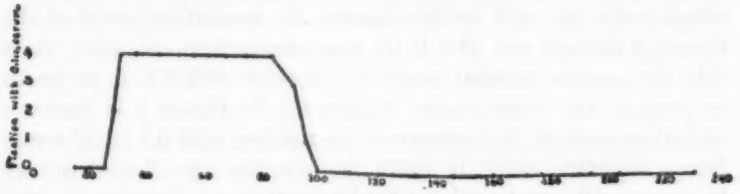

FIG. 1. BEHAVIOR OF WASSERMANN REACTION IN SYPHILITIC RABBIT INOCULATED INTRATESTICULARLY

disease, or 53 days after a lesion had been detected at the site of inoculation. As will be seen from the curves, the reaction in this animal began to subside in the higher dilutions within 10 days after it had attained its maximum, but remained completely positive with 0.1 cc. of serum until some time between the 83rd and the 108thday of the disease. The clinical lesions began to heal at almost the same time that the reaction began to diminish in the higher dilutions.

In our experience the tendency has been for the reaction to become negative spontaneously about 100 days after inoculation and to remain so thereafter in spite of clinical relapses in the inoculated testis or the occurrence of generalized lesions other than a metastatic orchitis. Transfer of popliteal lymph nodes, from animals whose reactions have thus become negative, to normal rabbits gave rise, in every instance

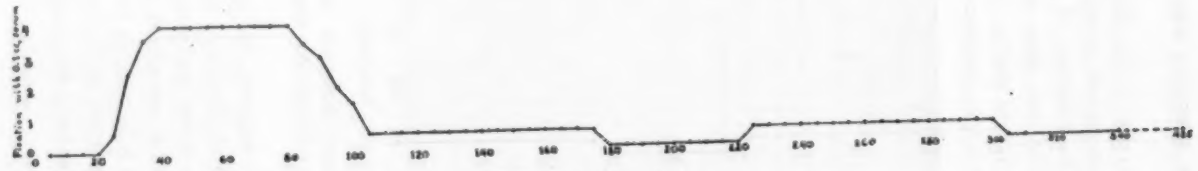

FIG. 2. COMPOSITE CURVE OF WASSERMANN REACTION IN 75 SYPHILITIC RABBITS

where tested, to infection in the latter, indicating that a negative reaction in the rabbit does not exclude latent syphilitic infection.

b. Wound inoculation. The behavior of the reaction in rabbits inoculated in this manner did not differ materially from that observed in those inoculated intratesticularly. Its appearance was somewhat delayed because of the longer incubation period of the primary

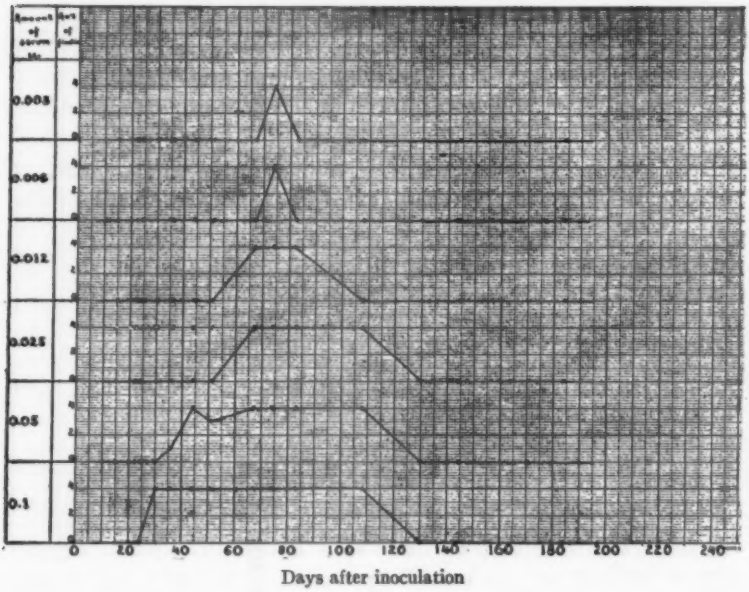

FIG. 3. BEHAVIOR OF WASSERMANN REACTION WITH SUCCESSIVE DILUTIONS OF SERUM OF SYPHILITIC RABBIT

lesion when produced in this manner, but once fully established it remained positive for as long as the lesion was present and in some instances for weeks after the lesion had healed spontaneously, even in the absence of any detectable metastatic lesions. With this particular method of inoculation it is possible to produce comparatively large chancres in a large proportion of the animals. The impression was obtained that, roughly speaking, the intensity and duration of a

positive reaction were directly proportional to the size of the lesion. Where the lesion was small, the time intervening between inoculation and the appearance of the positive reaction was longer and the duration of the latter less than when large primary lesions were produced. In general, no matter which of the two methods of inoculation was employed, the reaction paralleled remarkably closely the clinical course of the initial lesion, when the infection was allowed to take its course without alteration from the outside. In every instance in which a rabbit was inoculated with material known to contain treponemes, and a macroscopic lesion developed at the portal of entry, the reaction became positive. We have no observations upon untreated not reinoculated animals in which infection was produced without any local lesion at the portal of entry, and it will be of interest to determine whether such animals acquire a positive reaction. In general, our experience with the Wassermann test in the untreated syphilitic rabbit has been in close agreement with that of Nichols, Wakerlin and Carroll, and also Takenaka.

BEHAVIOR OF THE WASSERMANN REACTION IN ALTERED SYPHILIS OF THE RABBIT

When the usual course of the infection was interfered with either by operative removal of the primary lesion or by treatment with arsphenamine, marked variations from the usual behavior of the Wassermann reaction were encountered. The promptness with which the change occurred after the procedure had been carried out left no doubt as to the causal relationship between the two. The course of the reaction was studied in 38 castrated animals, and in 76 animals treated with arsphenamine. The results of the observations will be discussed in the order named.

Castration. When the testis originally inoculated was excised shortly after the reaction had become positive, the latter was at once reversed to negative. If there had been a wide excision of testicular material and no recurrent syphilitic lesion developed at the operative site and no metastatic lesion appeared in the opposite testis, the reaction remained negative thereafter. On the other hand if, as not infrequently happens in the case of the Nichols strain, a syphilitic lesion developed in the operative wound or a metastatic orchitis

occurred after castration, then the reaction almost always became positive again and from that time on paralleled the clinical course of the recurrent lesion whether it developed in the wound or in the other testis (see Fig. 4).

Castration before the reaction had become positive gave rise to practically the same results. If no further lesions developed in the operative wound or in the opposite testis the reaction remained negative thereafter, even though generalized lesions involving the skin, bone or eyes made their appearance later. If syphilitic lesions developed in the operative wound or in the opposite testis the reaction

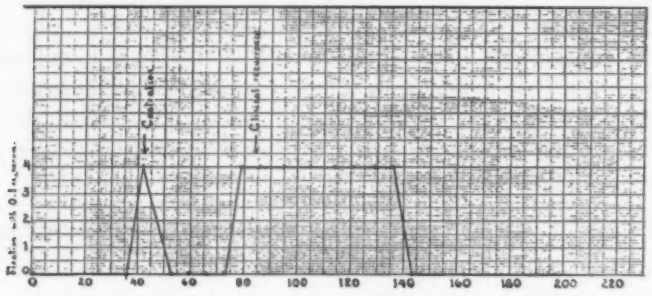

FIG. 4. EFFECT UPON WASSERMANN REACTION, OF REMOVAL OF SYPHILITIC TESTIS AFTER THE REACTION HAD BECOME POSITIVE

became positive after the appearance of these lesions and remained so as long as they were present (see Fig. 5).

Effect of treatment with arsphenamine. The effect of treatment with arsphenamine on the reaction varied to some extent depending upon the time in the course of the disease at which treatment was begun. All animals received the same dose of the drug intravenously, namely 10 mg. per kilo; hence they are strictly comparable as regards treatment. In the animals in which treatment was begun a short time after the appearance of the first positive test (41st day of disease), the reaction became negative with remarkable rapidity, even within 3 to 4 days after a single dose of the drug. When the treatment was postponed to a later date (110–168 days after inoculation), the re-

versal to negative was not so rapid and in several instances the reaction did not become negative until after a second dose of the drug had been administered, that is to say, as long as 3 weeks after the initial dose had been given.

Evidence of a provocative Wassermann reaction was encountered in 2 rabbits treated late in the course of the disease (145 and 163 days after inoculation). In both of these animals the reaction was negative at the time of the first injection. Following the administration of a single dose of arsphenamine the reaction became completely positive in each. After further treatment it became negative and remained so thereafter.

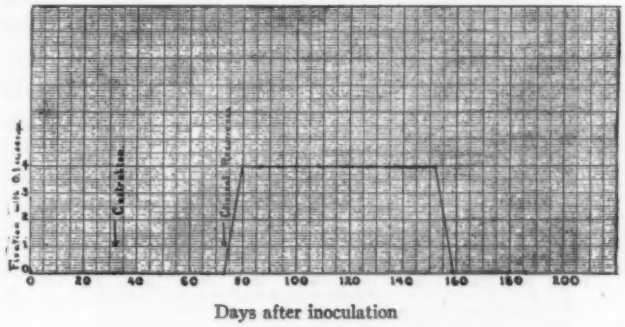

FIG. 5. EFFECT UPON WASSERMANN REACTION, OF REMOVAL OF SYPHILITIC TESTIS BEFORE THE REACTION HAD BECOME POSITIVE

Reinfections. Of considerable interest were the results obtained in rabbits treated at various stages in the disease and reinoculated with the homologous strain of *T. pallidum.* To some of these animals allusion has already been made in another communication (17), in which it was shown that animals treated early in the course of the infection were later susceptible to a second (intratesticular) inoculation with the homologous strain of treponemes, whereas those treated late in the course of the disease were refractory. Thus, in a group of 8 animals in which treatment was begun on the 41st day of the disease, the Wassermann reaction became positive after reinoculation in every instance, although no lesions developed at the site of reinoculation in

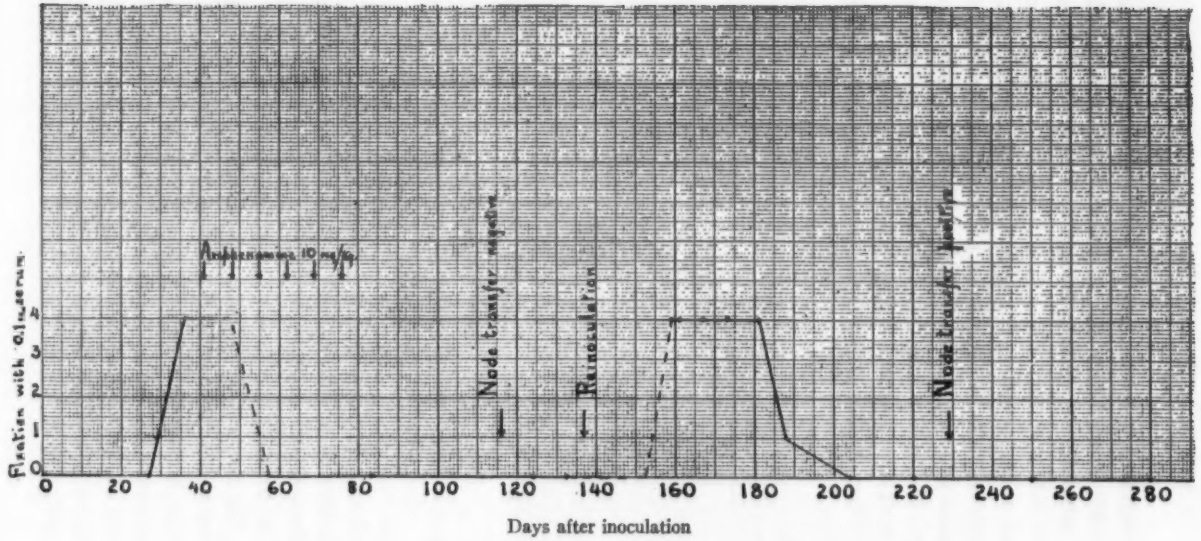

FIG. 6. BEHAVIOR OF WASSERMANN REACTION IN TREATED REINOCULATED RABBITS

6 of the animals. Study of the infectiousness of the popliteal lymph nodes of these reinoculated animals indicated that a second infection had been produced and that generalization of the virus had occurred in all but one of the eight rabbits, since the nodes after treatment but before reinoculation were shown to be incapable of transmitting the infection to normal animals, whereas after reinoculation they were shown to be infectious in every instance but one, even though in only 2 animals had a lesion developed at the site of reinoculation. The behavior of the reaction in one of the animals of this group in which treatment was begun on the 41st day of the disease and in which reinoculation was successful in producing a second infection is shown in Figure 6.

The behavior of the reaction in the group of rabbits treated 41 days after inoculation and then reinoculated by intratesticular injection was in striking contrast with the course of the reaction in the group in which treatment was begun late (193 days after inoculation) and reinoculation was carried out in the same manner. Without exception all of the animals in this group were refractory to a second inoculation and in no instance did a positive reaction make its appearance after reinoculation. Moreover, in none of these animals was it possible to demonstrate the presence of virus in the lymph nodes following reinoculation.

In general the occurrence of a positive Wassermann reaction in treated reinoculated animals was found to parallel very closely the occurrence of infection in the lymph nodes. Altogether, we have been able to collect, from several different experiments dealing with reinfection in syphilitic rabbits, observations upon 38 rabbits treated at different times and reinoculated in different ways, in which parallel determinations of the infectiousness of the lymph nodes and of the behavior of the Wassermann reaction were made after reinoculation. The relation of these two phenomena to one another is shown in Table I.

A study of Table I shows that, in 38 treated syphilitic rabbits which were reinoculated in different ways, there were 15 instances in which following reinoculation the Wassermann reaction was positive and the nodes were infectious at the same time. In addition there were 16 instances in which the reaction was negative and the nodes were sterile.

On the other hand, in 2 instances the reaction became positive but the nodes were non-infectious, and again in 5 instances the reaction remained negative, whereas the nodes were infectious. It happened that all the animals falling in the last 2 groups were reinoculated by deposition of the virus emulsion upon the surface of a granulating wound on the back. Further details of these particular animals are presented in the following paper of this series.

A study of the table also shows that in treated reinoculated syphilitic rabbits there is a close parallelism between the occurrence of a positive Wassermann reaction after reinoculation and the infectiousness of the popliteal lymph nodes on the one hand, and a negative reaction and sterility of the nodes on the other. The number of instances in which this parallelism was manifest was 31, or 81.5 per cent, as against 7 in-

TABLE 1

Relation of Wassermann reaction to infectiousness of popliteal lymph nodes in treated reinoculated rabbits

	NUMBER OF CASES	PER CENT
Wassermann positive, nodes infectious	15	39.4
Wassermann negative, nodes sterile	16	42.1
Wassermann positive, nodes sterile	2	5.2
Wassermann negative, nodes infectious	5	13.1

stances (18.3 per cent) in which it was not present. In those instances in which the nodes were found to be infectious but a positive reaction was not encountered, one cannot but wonder if perhaps a transient positive reaction had not developed but had been missed because too long an interval had elapsed between individual tests, since in some instances tests were not carried out oftener than every 3 or 4 weeks.

Takenaka found that in reinoculated rabbits the Wassermann reaction became positive if lesions developed following the second inoculation. Our experiments demonstrated that under certain conditions, such as early treatment, a positive reaction might occur following reinoculation and persist for several weeks even though no lesion made its appearance at the site of reinoculation, although there was evidence of infection and dissemination of the virus. These observations suggest that the occurrence of a positive reaction in a

treated syphilitic rabbit that has been reinoculated is strong evidence that the animal has been successfully reinfected even if no lesion develops at the site of reinoculation. Failure of the reaction to appear under these circumstances, however, cannot be taken as proof that reinfection has not taken place.

SUMMARY AND CONCLUSIONS

In general, observations upon a large series of rabbits in which the Wassermann reaction has been performed in accordance with a prescribed technic would tend to show that under the conditions described the reaction is almost never positive in normal rabbits or in rabbits with various infections to which the species is subject. Inoculation of rabbits with non-syphilitic material from human sources has in our hands not given rise to a positive test. On the other hand, inoculation of animals with syphilitic tissue or fluids direct from human sources has been followed by the appearance of a positive reaction in every instance in which a lesion containing treponemes appeared at the site of inoculation. Similarly, animals inoculated with material containing treponemes that have been carried through many series of rabbits have in each instance shown a positive reaction, if disease was produced at the portal of entry. In general the reaction parallels remarkably closely the clinical course of the primary lesion, becoming positive shortly after this is manifest and remaining so as long as the primary lesion is present. It tends to disappear when the primary lesion heals spontaneously, although the animal remains infected. In exceptional instances it remains positive long after the primary manifestation has healed spontaneously, and in such cases the animal is very apt to exhibit the malignant type of the disease, having many clinical relapses over a period of months. The occurence of generalized lesions of skin, bone or eye is, as a rule, not accompanied by the occurrence of a positive test. Operative removal of the primary focus of the disease may result in the failure of the reaction ever to appear, or in the postponement of its appearance. Treatment with arsphenamine in doses of 10 mg. per kilo administered intravenously brings about a prompt reversal of the reaction to negative. In exceptional instances it leads to a provocative reaction. When treated animals are successfully reinfected, with or without the pro-

duction of a lesion at the site of reinoculation, the Wassermann test becomes positive in a large proportion of the cases. It would thus appear to be a valuable guide in reinoculation experiments, although it cannot be accepted as an absolute criterion of reinfection.

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THE ORIGIN OF THE PHAGOCYTIC CELLS IN THE RABBIT[1,2,3]

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Investigators who have studied the fate of particulate substances injected intravenously all agree that these substances are more or less rapidly removed from the bloodstream and deposited in the lungs, liver, spleen, and in certain other organs of the body, where they are found later within phagocytic cells. There are essentially two schools interpreting the origin of these cells. According to Metchnikoff, Cohnheim, Ranvier and others, these are emigrated blood leucocytes which have undergone certain transformations in the tissue. On the other hand, Mallory teaches that all of the phagocytic blood cells are endothelial leucocytes and are derived primarily from the endothelium. While some of them may always be found in the circulation, their number may be greatly increased in certain pathological conditions. Aschoff and his followers maintain that these cells are derived from the reticulum and endothelial cells of the various organs, where they may remain fixed, or migrate away, or be carried by the blood stream as blood histiocytes. The reader is referred to Wislocki (1923), Maximow (1924), Aschoff (1924), and Foot (1925) for excellent reviews of the subject.

In a recent investigation Lewis (1925a) has demonstrated *in vivo* that the phagocytic cells in the lung of the frog originate from leucocytes of the circulating blood and not from endothelium nor from showers of macrophages from liver and spleen. So far the transformation of mammalian leucocytes *in vivo* into clasmatocytes in the

[1] I wish to express my thanks to Mrs. W. H. Lewis and to Dr. W. H. Lewis, in whose laboratory this work was done.

[2] The expenses for this investigation were paid from the Sharp and Dohme Fund of the School of Hygiene and Public Health, Johns Hopkins University.

[3] Submitted for publication July 6, 1926.

tissues, epithelioid cells in the lungs, splenocytes in the spleen, Kupffer cells in the liver, and so on, has not actually been demonstrated, but tissue cultures furnish strong presumptive evidence that such transformation does occur. M. R. Lewis (1925b) and Lewis and Lewis (1925) have watched the formation of macrophages and epithelioid cells from ordinary leucocytes in incubated blood, and M. R. Lewis (1925c) has shown that leucocytes may develop into cells indistinguishable from Kupffer cells in cultures of the liver.

In the experiments here reported, the particulate matter was introduced into the bloodstream where it was rapidly taken up by the leucocytes. Some of these marked leucocytes were injected into another animal and the fate of the cells was followed in the bloodstream and tissues of both the new host and of the original animal. In this way it has been possible to show that it is the circulating leucocytes which phagocytose particulate substances; that they stop out in the capillaries of the lungs, liver, spleen and other tissues and there become transformed into the cells which are usually interpreted as composing the reticulo-endothelial system.

MATERIAL AND METHODS

Rabbits were chosen as the experimental animal because they have been most commonly used by other investigators and are easy to inject intravenously and to bleed by heart puncture. In a microscopical study of the organs of normal rats and rabbits there is often discovered a striking amount of black particles in the phagocytic cells, not only in the lungs, but in the liver, spleen and connective tissue. This proved to be a confusing factor when small injections of carbon were introduced intravenously in an attempt to trace the fate of injected particulate substances in the body. Powdered carmine was finally selected as a suitable material for marking the cells. About 0.1 gram of the dry powder (carmine rubr. Gram) was ground in a mortar for a few minutes and then 3 cc. of distilled water were added gradually. The suspension was sterilized by boiling, and centrifuged for ten minutes to remove clumps and large particles; it was considered satisfactory when the particles were less than 1μ in diameter and there were no clumps present. From 1 to 2.5 cc. were used for each dose. The intravenous injection of distilled water

causes some hemolysis but no symptoms in the rabbit in the amounts used. Suspensions in physiological salt or Locke solution, however, clumped too readily in the test-tube to be satisfactory. The carmine suspension in distilled water apparently clumped very little in the bloodstream. In the experiments with Higgins' India ink, 1 cc. of a 1–10 dilution in distilled water was used. This suspension clotted considerably, forming fairly large particles and some threads.

Injections were made into the marginal ear vein or into the heart during a period of two minutes. Five minutes later blood was withdrawn from the heart into a large syringe containing 1 mg. of heparin to 20 cc. of blood to prevent clotting. From this a few whole blood cultures were made for immediate examination, two smears for Wright's stain, and 5 cc. centrifuged for white blood cell cultures in plasma. The remainder of the blood was emptied into sterile tubes and taken up again into sterile 10 cc. syringes for reinjection into the ear veins of other rabbits.

Cells were marked outside of the animal body also by drawing blood into syringes containing the carmine and heparin, centrifuging and washing the white blood cells and resuspending them in Locke solution.

CARMINE INJECTIONS

The first rabbit received 2.5 cc. of carmine in the heart under ether and was bled from the heart five minutes later. The hanging-drop blood cultures showed no free carmine, while most of the mononuclears and polymorphonuclears contained some, usually only a few grains in each cell. There were none in the eosinophiles or the small lymphocytes. A study of the Wright stain indicated that the total number of white blood cells in the circulation had decreased considerably. Werigo (1892) and others have called attention to the leucopenia which follows the intravenous injection of particulate substances.

In the lungs numerous carmine-laden polymorphonuclear leucocytes and monocytes filled the capillaries. Some of these were plastered along the capillary walls and had much the appearance of endothelial cells, but if watched for several minutes often were seen to change their shape or to migrate away. There was no carmine in the large "dust" cells, in the epithelial cells, or in the endothelium.

Rabbit 2 received in the ear vein 9 cc. of the "marked" whole blood from rabbit 1 and was killed one hour later. In this case all of the carmine was already within white blood cells at the time of injection. Whole blood cultures, made just before death, revealed the fact that many of these injected cells containing carmine were still circulating in the blood stream at the end of an hour. However, by far the largest proportion had lodged in the capillaries of the lungs, where a picture identical with that found in rabbit 1 was presented. The cells containing carmine were unmistakably polymorphonuclear leucocytes and monocytes, though many of them were flattened against the capillary wall and often difficult to distinguish from it unless watched long and carefully. No carmine cells were found in the liver or lymph nodes, but several mononuclears in the spleen contained a few grains each. The Wright stain showed an apparent increase in the number of white cells and many platelets. In this rabbit, as in the previous one, all of the carmine cells were confined within the capillaries and sinus spaces. Moreover, the cells which lodged out were those containing the largest amount of carmine, and only the cells with a few of the smallest grains remained in the circulation.

The third rabbit had received only 3 cc. of blood and was examined after twenty-four hours. There was now no carmine in any of the circulating cells. In the lung the carmine was found mostly in mononuclears, some of which were quite large, with flattened nucleus. A few large mononuclears containing carmine were found in the liver and in the spleen. These cells were located along the endothelial linings as well as in the tissue spaces. Rabbits that received the carmine suspension directly instead of the marked cells often had many carmine cells in the circulation after twenty-four hours. This was determined by centrifuging 10 cc. of blood (containing 0.5 mg. of heparin) and making 10 to 20 cultures of the buffy coat in autoplasma. In one rabbit over half of the polymorphonuclears and monocytes each contained a few small grains of carmine. In other rabbits only an occasional cell with fine carmine granules was seen. As would be expected, there were always more cells with carmine in the capillaries and tissues in these rabbits than in those receiving the marked cells, but the distribution and appearance of the cells in both were identical.

Rabbit 4 which had received 3 cc. of blood from the first rabbit was examined at forty-eight hours. In this animal the spleen, lungs, liver and coeliac lymph glands contained a few large mononuclear cells with carmine, along capillaries and in the tissues beyond. No carmine cells were found in the bloodstream either in the "marked cell" or directly injected rabbit.

On the fifth day rabbit 5, which had received 5 cc. of blood, was killed, and carmine was found in the lungs, liver, spleen and coeliac lymph nodes. In the lungs it was contained in large epithelioid cells, in the spleen in splenocytes and macrophages, in the liver in Kupffer cells, and in the coeliac lymph nodes in large hypertrophied mononuclears. In other words, the carmine was now deposited in the so-called reticulo-endothelial system. A similar picture was presented in the organs of rabbits injected five days previously with the carmine suspension alone.

CARBON INJECTIONS

Foot (1925) claims that colloidal carbon (Higgins' India ink) behaves differently from mechanical suspensions and that it is taken up by vascular endothelium as well as by "specific endothelia." For comparison with the animals injected with carmine, a rabbit was injected with 1 cc. of a 1–10 dilution of India ink. The results obtained were entirely comparable with those observed in the carmine injections, except that the polymorphonuclears contained far less of the carbon. Most of the circulating mononuclears and a few polymorphonuclears had ingested carbon particles five minutes after the injection. There was also some free carbon which had clotted in the plasma. At two hours, and even at twenty-two hours after the injection, there were many circulating mononuclears containing carbon. The number of carbon cells was noticeably decreasing and those remaining contained only a few of the very small granules. In the lungs, especially, and also in the liver and spleen, carbon-laden mononuclears were found in the capillaries and, after twenty-four hours, in the tissue spaces of the lungs and in the reticulum of liver and spleen. There was no carbon in the endothelial cells. In the rabbit examined ten days later the carbon-laden cells had hypertrophied into large epithelioid cells in the lungs, into Kupffer cells in the liver,

and into characteristic splenocytes and macrophages in the spleen. Some of them contained the debris from dead cells while others had apparently ingested little else.

Thus, by using a small dose and a weak solution, it becomes perfectly clear that the circulating leucocytes phagocytose the carbon just as they do the carmine granules and that they afterward lodge out in the capillaries of the various organs. Wislocki and Foot used at least 5 cc. of a 50 per cent dilution of Higgins' India ink and noticed that there was first a "filtering out" of the carbon in the organs, where it was later taken up by phagocytic cells. Such an enormous dose floods the capillaries with granules and causes numerous emboli of sticky carbon-laden cells, free particles piling up around them, so that the true picture is entirely obscured and phagocytosis does not *appear* to play a prominent part in the initial deposition of the carbon.

In our experiments with small injections identical results were obtained using either a mechanical suspension of carmine or a colloidal solution of carbon, or cells "marked" with carmine in the body of one animal and injected intravenously into another animal. In every case there is a lodging out of cells which have already ingested material rather than a subsequent phagocytosis of lodged out particles. The latter may occur when the dose is large or the injected substance clumps in the blood stream and becomes caught in the capillaries.

BLOOD CULTURES

Cultures of the buffy coat from 10 cc. of blood taken from animals injected with carmine were made in normal plasma five minutes, one hour, and twenty-four hours after injection. In these cultures not only the mononuclears containing carmine, but all others as well (except the small lymphocytes), were observed to hypertrophy and develop into typical clasmatocytes and epithelioid cells, identical in appearance with many of those found in the lungs, liver, spleen and connective tissue of the animal body. There is also some indication that a few polymorphonuclears may undergo a similar transformation (W. H. Lewis, 1926). Many of those in the cultures round up and die, and are phagocytosed by other cells; others survive unchanged

almost as long as the culture, and still others apparently lose their distinctive granulations, accumulate fat and plasma granules, and hypertrophy like the mononuclears. All stages are found between the typical polylobed nucleus, with diffuse nucleolar material, of the granulocyte, and the round or oval, vesicular nucleus, with well defined nucleolus, of the clasmatocyte.

DISCUSSION

By the use of marked cells, it has been possible to follow in the living animal the same transformation of blood cells into macrophages, clasmatocytes and epithelioid cells as occurs in tissue cultures. First of all, we know that the phagocytic cells that ingested the carmine in the first rabbit were circulating blood cells, because nearly all of the polymorphonuclears and monocytes in the sample of blood withdrawn from this animal contained the carmine. If these cells were a "shower" from the spleen following the injection, as suggested by Simpson (1922), where and why did the circulating leucocytes disappear so rapidly? That many of them did disappear is certain, because of the leucopenia following the injection, and examination of the lungs, liver and spleen shows that they left the blood stream laden with carmine. Further proof that they are not special phagocytic cells from the hematopoietic organs, responding to the injections, is furnished by the experiments in which the cells were marked outside of the body by being drawn into a syringe containing the carmine suspension. That much of the phagocytosis may occur in the slower capillary beds is no doubt true, but the couclusion that it is accomplished by the circulating leucocytes rather than by the endothelium of the capillaries and sinuses, as claimed by Foot, seems inevitable. The endothelium could not possibly proliferate within five minutes to provide the number of cells observed; and moreover, the phagocytic cells, when examined within five minutes after the injection, are unmistakably blood cells. Even after twenty-four hours they are still recognizable, though not as typical as before. After three days many of the mononuclears have hypertrophied into cells ordinarily considered typical of the tissue phagocytes. The nuclei contain definite nucleoli and there are more numerous cytoplasmic granules which stain with neutral red, and which are now less uniform

in size and more irregularly distributed. By the fifth day many of these cells have phagocytosed other material, including red blood cells, dead polymorphonculears, or other tissue debris. Even during the first hour after an injection, a few of these cells in the liver or spleen may contain carmine. They are the pre-existing clasmatocytes, are highly phagocytic and ingest anything which comes into contact with them. It might be argued that the blood cells containing carmine are always ingested by pre-existing clasmatocytes rather than that there is a *transformation* of the blood cells into clasmatocytes. But against this we have the evidence of successive stages of the transformation in the living tissue and the demonstration of actual and continuous transformation in cultures of the white blood cells from the peripheral circulation.

CONCLUSIONS

By means of the intravenous injection into the rabbit of white blood cells which have ingested carmine particles, it is possible to produce the same picture in the organs of this rabbit as occurs in those of a rabbit which has received an injection of carmine particles directly. This method shows that the so-called reticulo-endothelial system, *i.e.*, the system of phagocytic cells located in various organs of the body, is made up largely of transformed white blood cells which have left the blood stream because they have become abnormal, either through phagocytosis or through some injury.

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BACILLUS OZAENAE FOETIDAE, PEREZ, AND BACILLUS PROTEUS IN OZAENA[1,2]

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INTRODUCTION

Ozaena is a chronic disease of the nose characterized by a mucopurulent discharge, crusts, a specific fetid odor, and atrophy of the turbinate bones. Following the fundamental work of Pasteur, many attempts were made to discover the virus of this disease, inasmuch as the dystrophic, syphilitic, and nervous theories of its etiology were unsatisfactory. Significant bacteriological study began with Loewenberg and Abel. Abel, in 1893, and Loewenberg, in 1884 and 1894, found a bacillus very similar to Friedländer's bacillus in every case of ozaena. They called it "Coccobacillus ozaenae" and described it as capsulated, Gram negative, and non-motile, but differing in some particulars, especially in its carbohydrate fermentations, from the typical *B. pneumoniae* of Friedländer. Subsequently various workers identified this bacillus with that of Friedländer, while others thought it similar to *B. rhinoscleromatis* of Fritsch, a bacillus considered by some to be the cause of rhinoscleroma, a distinct clinical condition of the nose in which the latter is always found. Others considered the bacillus of Abeland Loewenberg closely related to *B. capsulatus mucosus* of Fasching. Although Loewenberg and Abel did not succeed in reproducing nasal disease or any of the symptoms of ozaena by infecting animals with their bacillus, its close relationship to *B. rhinoscleromatis* and its frequent occurrence in the typical or atypical cases of ozaena led them, in spite of their lack of further proof, to consider it as the virus of ozaena. In 1899 Perez described a new bacillus as the pathogenic agent of ozaena and

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called it "*Coccobacillus foetidus ozaenae.*" He isolated the same bacillus from 7 out of 11 typical cases. From 11 cases without fetor, which he also considered to be ozaena, he isolated another bacillus similar to the first, which differed only in producing in cultures an odor which was very slight, or none at all. The "*Coccobacillus foetidus ozaenae*" is described by Perez as a non-motile, Gram-negative, pleomorphic coccobacillus, sometimes with long filamentous forms in the same stained film. It grew well in all ordinary media, producing the characteristic smell of ozaena, especially in infusion broth and infusion agar. Infusion broth, after 24 hours' incubation, became turbid, with a heavy "filamentous" sediment but no pellicle. The colonies on agar were thick, transparent and glistening, and on potato thick and yellowish. It formed indol but did not liquefy gelatin, and did not coagulate or digest milk. It was pathogenic for white mice, guinea-pigs and rabbits. Inoculated subcutaneously, it produced an abscess; intraperitoneally in the guinea-pig, a hemorrhagic peritonitis; intravenously in the rabbit, it killed in 24 to 25 hours. Some of the rabbits, surviving for 4 to 5 days, developed a yellow-green mucopurulent nasal discharge. Then they began to emaciate and died after 10 days to 4 months. At autopsy they showed a greatly enlarged spleen, pericarditis or peritonitis "exudativa," and congestion of the cribriform plate of the ethmoid, which was covered with a thick, yellowish "mucous" exudate. From the nose Perez recovered the same bacillus. One rabbit became emaciated and after a month presented symmetrical necrosis of the ears. After 4 months an abscess appeared on the tip of the nose, from which Perez cultivated his bacillus. After 6 months the rabbit was killed and the anterior and posterior turbinate bones were found to be diminished in size and atrophied. In a second paper (cited by Shiga, 1922) Perez reported that some of his strains were slightly motile and that they liquefied gelatin. Perez isolated the same *Bacillus ozaenae* from the nose of a dog and suggested that the dog is the source of infection for human beings by direct contact. Horn and Victors, in the United States, thought that *B. bronchisepticus*, isolated from a dog's nose, and *Coccobacillus foetidus* Perez, were markedly similar and proposed to unite them in the same "Hemorrhagic Group" under the name "Rhinosepticus." Ferry, Noble and others showed con-

clusively that *B. bronchisepticus* is quite distinct from the Perez bacillus and has no relation to ozaena.

Hofer (1913) found the bacillus described by Perez in some cases of ozaena and succeeded in producing similar lesions in the rabbit, confirming the work of Perez.

Ward (1917) and Ward and Beaver (1918) studied 50 cases of fetid atrophic rhinitis, which led these authors to consider members of the Perez and Proteus group as responsible for the malodor of ozaena. They supplemented this with a study of 21 cases of atrophic rhinitis without odor and found *B. mucosus capsulatus* as the preponderating organism, while the Perez and Proteus groups were found in only a small percentage of cases. *B. ozaenae* was either motile or non-motile, Gram-negative, produced slow fermentation of glucose and a characteristic pigment in gelatin, and decomposed proteins with the formation of mercaptan, H_2S, and ammonia.

Shiga (1922), repeating the work of Perez and Hofer, obtained similar results. He never produced the same picture with the Abel-Loewenberg bacillus as with the Perez bacillus, and does not believe that the Abel-Loewenberg bacillus has any relation to ozaena. Of his 50 strains of the non-motile, non-liquefying Abel-Loewenberg bacillus, none produced the characteristic odor of the Perez bacillus. He isolated 20 strains of the Perez bacillus, the majority of which were motile, liquefied gelatin more or less slowly, and coagulated milk. The more rapid the liquefaction of gelatin, the more rapid was the coagulation of milk. The liquefying strains he called *B. ozaenae liquefaciens*. He considered the Perez bacillus to constitute a very large group, most of the strains motile, some non-motile. In broth they usually grew without a pellicle. They were pathogenic for rabbits, producing symmetrical necrosis of the ears, a pathological action considered by Shiga as the most significant proof of the specificity of the *Coccobacillus foetidus* as the virus of ozaena. When the rabbits died, following intravenous injection, the autopsy showed a thymus congested and turgid with blood, congestion of the lungs, nose and Peyer's patches, and sometimes a hemorrhagic exudate into the pericardium or peritoneum. Shiga produced 4 distinct immune serums. Organisms of any one strain were agglutinated by one or two of the four serums, never by all. The sugar fermentations varied.

Three typical Perez strains are listed which do not ferment maltose (p. 526), while thirty strains are recorded (p. 532) as fermenting maltose as well as saccharose. This description of Shiga indicates a liquefying Perez bacillus, morphologically and culturally very similar to the Proteus group. He agglutinated Proteus X^{19} with agglutinins made with a non-motile, non-liquefying Perez strain, "AC^{29}", in one case, and with a motile, liquefying Perez strain, "AB^{13}," in the other slightly (±) at 1/250 and not at all (−) at 1/500.

In 1923 Shiga published a second paper, in which he reported having produced a toxin, as had others before him, from his non-liquefying and liquefying strains of the ozaena bacillus. The toxin was very labile and strongest in broth filtered after 4 to 7 days' incubation. One-half to 2 cc., injected intravenously, killed a rabbit of 1 to 2 kilos in 2 to 6 days, producing the same lesions as the live organisms. Shiga thought that death was produced by a toxin with an affinity for the bloodvessels and nerves. He was not able to produce an antitoxin by injecting the toxin. He considered the liquefying strains to be very different from *Bacillus proteus*, because of a difference in pathogenicity for rabbits.

EXPERIMENTAL

We have studied 28 typical cases of Ozaena foetida in Europe and the United States. For the isolation of the Perez bacillus we adopted the method of Perez, inoculating some of the nasal discharge into 10 to 12 tubes of infusion broth and closing them with a rubber stopper. After 24 or 48 hours all tubes were opened, and those giving the characteristic odor were inoculated for the isolation of pure cultures into Drigalski and infusion agar. We found, as have others, that it is sometimes very difficult to isolate the Perez bacillus. Usually, when we found a tube with a characteristic odor, the predominating bacillus was Gram-negative. Frequently we found a motile bacillus of the Proteus group, non-pathogenic for rabbits. Most of the non-motile bacilli were of the Abel-Loewenberg type. The frequency with which the Perez bacillus is overgrown by the Abel-Loewenberg or other similar Gram-negative bacilli obliged us to modify the method of isolation. The primary broth culture showing the most marked specific ozaena smell was plated and, in addition, 4 cc. were inoculated

subcutaneously into a rabbit. Often we found in the blood and nose of the rabbit, dead of the inoculation, the same Perez bacillus which we fished from Drigalski or infusion agar plates. In two cases we did not succeed in isolating the Perez bacillus from the media, but found it in pure culture in the nose and blood of the rabbit. One isolation of Perez bacillus (Perez II) was non-motile, the second (Strain II) was motile and liquefied gelatin.

From the 28 cases we isolated only 7 Perez bacilli, 3 non-motile, 4 motile, all very pathogenic for rabbits. The percentage of isolations is smaller than that of other workers, perhaps because we cultivated, by our method of isolation, only those strains that were pathogenic for the rabbit and produced lesions described as characteristic for the Perez bacillus. It was our aim to study the relationship between typical Perez bacilli and atypical, liquefying Perez bacilli, and to compare them with typical *Proteus vulgaris*, and it was necessary to use strains pathogenic and toxigenic for the rabbit to enable us to study their pathogenicity, toxicity, toxigenic and serological properties. As Ward and others have shown, many species of bacilli are found in the ozaena discharge. Most of them are common inhabitants of the nose or may be secondary invaders. The bacilli most frequently found are *Proteus bacillus* or the Perez bacillus. Ward, in 50 cases of typical ozaena foetida, found 34 atypical Perez bacilli (Group II of Ward), 11 typical Perez bacilli, and 14 *B. proteus* or 59 bacilli of the Perez and Proteus groups in 50 cases; while in 21 cases of atrophic rhinitis he found 1 Proteus and 1 Perez bacillus. Ward made 150 examinations of his 50 cases. We were able to make only 34 examinations of 28 cases and found 7 bacilli pathogenic for rabbits and 9 non-pathogenic, or 55 per cent.

It seemed to us that the difficutly of isolating either typical non-liquefying or liquefying Perez bacilli from clinically typical cases of ozaena might be due not merely to overgrowth with other bacteria, but to some further factor; therefore we looked for some evidence of bacteriophage activity. From 5 cases of frank ozaena foetida in which the Perez bacillus was not isolated, we emulsified a large piece of nasal crust, inoculated it into broth and incubated for 24 hours. After filtration through infusorial earth and a Berkefeld filter it was tested against a typical Perez bacillus, a liquefying Perez bacillus,

and a *Proteus vulgaris*. Two cubic centimeters of a filtrate added to a young broth culture did not show any bacteriophage action against these bacilli by any method.

As others have pointed out, sometimes, and especially in old chronic cases, it is impossible to find the Perez bacillus, either typical or atypical, in the first or second cultivation, whereas the third may be successful. In the old chronic cases the bacillus may be hidden in the depths of the tissue, producing the persistent necrosis and fetid discharge.

All our strains are pathogenic for the rabbit, producing death, if inoculated intravenously in sufficient dose, in 24 hours, with the same pathological lesions as described by Perez, Hofer, Shiga, and others. Some of the strains produced symmetrical necrosis. All are toxigenic, producing a strong agglutinin and sensibilisin when injected into animals. Compared with a Hofer strain, which we obtained from Hofer in Vienna, 3 have the same cultural and morphological properties, while 4 have the properties of the motile *B. ozaenae liquefaciens* of Shiga. From other laboratories we obtained 5 strains of typical *Bacillus proteus vulgaris* and 1 of *B. proteus mirabilis*. One of the strains, Proteus II, was very pathogenic for the rabbit; injected intravenously, 1 to 2 cc. of an infusion broth culture produced death in 24 hours, with congestion and hemorrhagic lesions described as specific for the Perez bacillus. Proteus I was at first non-pathogenic for the rabbit. We passed it twice intraperitoneally throug hguinea-pigs and then intravenously through the rabbit, after which it became very pathogenic. One-half of a cubic centimeter of a 24-hour infusion broth culture, given intravenously, killed rabbits, with the congestive hemorrhagic lesions described as characteristic for the Perez bacillus, and was toxigenic, producing a toxin identical with that of Hofer's Perez strain and of our other Perez bacilli.

Fourteen strains were used in this study. Group I was made up of typical Perez bacilli, Hofer's Perez strain serving as a standard of comparison; our own ozaena isolations; "Perez II" and "Perez X," from Europe; and "Perez XI" from a patient in the Johns Hopkins Hospital Dispensary. Group II consisted of the strains in which we were especially interested, all similar to *B. ozaenae liquefaciens* of Shiga. All were from ozaena cases; "Strain I" was isolated in Europe

and "Strains II, III, and IV" from patients in the Johns Hopkins Hospital Dispensary. Group III consisted of strains from various laboratories, labelled as typical Proteus: "Proteus I" from the collection in this laboratory, obtained from Dr. Kraus in Vienna; "Proteus II," from the Bacteriological Laboratory of the Johns Hopkins Medical School; "Proteus III," isolated in this laboratory in 1919 from an infant's stool; and "Proteus IV," "Proteus V," and "*Proteus mirabilis*," coming from Dr. Rettger, of the Sheffield Laboratory of Bacteriology, Yale University.

MORPHOLOGY

All the strains are morphologically and tinctorially similar. They are Gram-negative, non-motile, without capsule or spores. The usual form is a short bacillus, 1 to 2 micra long by 0.8 micra wide, together with a few shorter coccobacilli and larger, flexible, filamentous forms, or streptobacilli. The coccobacillary forms are most abundant in agar cultures. The bacilli are evenly colored by all the common anilin dyes and present no irregular or metachromatic staining. In old cultures there are many poorly staining bacilli but few involution forms; this is characteristic of the Perez bacillus, as well as of *B. proteus vulgaris*. The only variation in the picture is presented by *Proteus mirabilis* of Group III, which has longer rods and develops involution forms.

Motility is not general. No typical Perez bacillus (Group I) is motile. Using the designations explained above, we may say that, in Group II, Strain I is only sluggishly motile, Strain II is very active, darting across the field, while Strains III and IV are less active. Group III also shows variation. Proteus I is very slowly motile Proteus IV is very active, while the motility of the others shows gradations between these extremes.

CULTURAL REACTIONS

In our cultural work we used all the common media. For the study of fermentation reactions we employed sugar-free infusion broth in vial tubes, sugar-free infusion agar, and Barsikoff's medium, to each of which the sterilized litmus and test substance were added

before inoculation. The most regular and characteristic reactions were obtained in Barsikoff's medium with litmus and the test supstance sterilized in the Arnold or by boiling on three successive days for half an hour. Gelatin liquefaction was tested by cultivation at laboratory temperature or at 20°C.

All the strains grew well. In a number of media they produced identical reactions, allowing of no differentiation. Thus, all formed indol in peptone water and broth; all fermented levulose; all produced gas in neutral red dextrose agar, changing the red color to a fluorescent yellow-green; none fermented lactose, raffinose, inulin, dextrin, starch, dulcitol or manitol; none produced hemolysis in infusion agar plates to which rabbit whole blood or washed red cells had been added. Agar washed off with saline one or two days after heavy inoculation with any one of the strains failed to support a growth of any of the others.

In the other media there was a greater or less variation, and these differences, particularly in gelatin liquefaction and in fermentation reactions, corresponded fairly closely to the grouping according to the source of the material. These are summarized in Table I.

Infusion broth. All cloud the broth. Turbidity is less with Group I. Pellicle formation is inconstant. From an initial pH of 7.8 the reaction changes in two days to pH 8 or 8.2, where it remains for twelve days.

Infusion agar. On agar they produce a luxuriant butyrous growth. Group I forms well-defined colonies, while in Groups II and III there are well-defined colonies if the medium is dry, but a spreading growth if it is moist. In stab cultures Group I strains grow more superficially.

Drigalski medium. Group I forms well-defined, translucent bluish colonies. Groups II and III form bluish white, thicker, denser colonies, with a tendency to spread, which is less pronounced than on infusion agar.

Lead acetate agar. Group I strains show a slight brown in the surface growth. Groups II and III develop spreading brown colonies.

Potato. All grow luxuriantly. Of Group I, Perez II remains grayish white, while the others become brown in about 10 days; Groups II and III, brownish gray in 5 to 10 days.

TABLE I

Cultural reactions

		MOTILITY	MILK	LITMUS MILK	GELATIN	NEUTRAL RED DEXTROSE AGAR	LOEFFLER'S SERUM	LEAD ACETATE AGAR	POTATO
Group I	Perez Hofer	+	− 30d	−	−	+	− Gray white	±	Brownish
	Perez II	−	− 30d	−	−	+	− Gray white	±	White
	Perez X	−	− 30d	−	−	+	− Gray white	±	Brownish
	Perez XI	−	− 30d	−	−	+	− Gray white	±	White
Group II	Strain I	+	+ 5d	r +	+	+	+ Brownish	++++	Brownish
	Strain II	+++	+++ 4d	r ++	++	+	+ Brownish	++++	Brownish
	Strain III	++	++	r ++	+++	+	+ Brownish	++++	Brownish
	Strain IV	+	+	r ++	+++	+	+ Brownish	++++	Brownish
Group III	Proteus I	+	+ 20d	r 4d + 20d	+++	+	− Brownish	++++	Brownish
	Proteus II	++	+ 20d	r 10d + 20d	++	+	+ Brownish	++++	Brownish
	Proteus III	++	++ 20d	+ 20d	+ 10d	+	− Brownish	++++	Brownish

Group III	Proteus IV	++++	− 30d	− 30d	+	+	− Gray white	++++	Brownish
	Proteus V	+	− 30d	− 30d	+	+	− Gray white	++++	Brownish
	Proteus mirabilis	++	++ 8d	++ 8d	+++	+	− Gray white	++++	Brownish

+ in milk = coagulation and digestion. Loeffler's serum = digestion. − = no change.
Litmus milk = acid. Lead acetate agar = blackening. d = day.
Gelatin = liquefaction. Neutral red dextrose agar = fluorescence and gas. r = reduction of litmus.

		BARSIKOFF MEDIUM													
		Levulose	Glycerol	Maltose	Dextrose	Galactose	Xylose	Saccharose	Dextrin	Mannitol	Dulcitol	Raffinose	Lactose	Inulin	Starch
Group I	Perez Hofer	+	+	+	+	+ 6d	−	−	−	−	−	−	−	−	−
	Perez II	+	+	+	+	+ 6d	−	−	−	−	−	−	−	−	−
	Perez X	+	+	+	+	+ 6d	−	−	−	−	−	−	−	−	−
	Perez XI	+	+	+	+	+ 6d	−	−	−	−	−	−	−	−	−
Group II	Strain I	+	++c	+ → − 20d	+	++c	++c	+ 10d	−	−	−	−	−		−
	Strain II	+	++c	+ → − 20d	+	++c	++c	+ 10d	−	−	−	−	−	−	−
	Strain III	+	+	+ → − 10d	+	++c	++c	+ 10d	−	−	−	−	−	−	−
	Strain IV	+	+	+ → − 10d	+	++c	++c	+ 10d	−	−	−	−	−	−	−
Group III	Proteus I	+	++c	+ → − 10d	+	++c	++c	+ 12d	−	−	−	−	−	−	−
	Proteus II	+	++c	+ → − 10d	+	++c	++c	+ 10d	−	−	−	−	−	−	−
	Proteus III	+	++c	+ → − 6d	++c	++c	++c	+ 10d	−	−	−	−	−	−	−
	Proteus IV	+	+	+	+	+	+	+ 8d	−	−	−	−	−	−	−
	Proteus V	+	++c	+ → − 10d	+	+	+	+ 4d	−	−	−	−	−	−	−
	Proteus mirabilis	+	−	++c	+	+	++c	−	−	−	−	−	−	−	−

+ = acid. − = alkaline. c = coagulation and precipitation. d = day.

TABLE I—*Continued*

		SMITH DURHAM BROTH TUBES									
		Glucose		Maltose		Dextrose		Galactose		Xylose		Saccharose	
			%		%		%		%		%		%
Group I	Perez Hofer	−	0	++	20	++	20	++	20	−	0	−	0
	Perez II	−	0	++	20	+++	30	++	20	−	0	+	10
	Perez X	+	10	++	20	+++	30	++	20	−	0	−	0
	Perez XI	−	0	++	20	++	20	++	20	−	0	−	0
Group II	Strain I	+	10	+	10	+++	30	++	20	+	10	+++	30
	Strain II	+	10	+	10	++	20	++	20	+	10	++	20
	Strain III	+	10	+	10	++	20	++	20	−	0	+++	30
	Strain IV	++	20	++	20	++	20	++	20	++	20	++	20
Group III	Proteus I	+	10	+	10	++	20	++	20	+	10	+++	30
	Proteus II	+	10	+	10	++	20	++	20	−	0	++++	40
	Proteus III	+	10	+	10	++	20	++	20	−	0	+++	30
	Proteus IV	−	0	−	0	++	20	−	0	−	0	+++	30
	Proteus V	−	0	+	10	++	20	+	10	−	0	++	20
	Proteus mirabilis	+	10	+	10	++	20	+	10	+	10	++	20

+ = acid.
− = alkaline.
% = gas, in inverted ampule.

Loeffler's blood serum. Growth is luxuriant and butyrous. Group I forms grayish white colonies and fails to digest. Group II digests in from 2 to 10 days and the colonies become brownish gray in from 4 to 10 days. In Group III, Proteus II digests, while the others fail to do so. The colonies of Proteus I, II, and III are brownish, whereas the others are grayish white.

Robertson's egg-cube medium. All strains cloud the broth and digest the egg-cube to a certain extent. Subsequently the broth clears, in 12 to 15 days, with a heavy sediment of bacteria and detritus from the egg. Group I shows the least turbidity, and on clearing leaves the broth lighter in color than before inoculation. Group II leaves a clear brownish supernatant fluid. In Group III, with Proteus I, III, IV, and V the fluid is paler, while with Proteus II and *Proteus mirabilis* it is brown.

Milk. Group I produces no change. Group II coagulates and digests in 4 to 5 days. In Group III Proteus I and II digest in 20 days, Proteus III in 30 days, and *Proteus mirabilis* in 8 days, while Proteus IV and V produce no change in 30 days.

Litmus milk. Group I produces no change. Group II decolorizes in 1 to 2 days prior to the digestion at 4 to 5 days. In Group III Proteus IV and V produce no change, while the other strains decolorize in from 4 to 10 days and begin to digest in from 8 to 30 days.

Gelatin. Group I produces grayish white colonies and fails to liquefy. Group II grows in characteristic grayish white, spreading, amoeboid colonies. In plates colonies appear in 1 to 2 days, showing an opaque center with prolongations in all directions, with changes in form after liquefaction begins. This varies in the time of onset and in rapidity. Strains III and IV liquefy half a tube in 24 hours. Strain I begins at 24 hours and liquefies half the tube in 3 days. Group III shows some variation. Proteus I and *Proteus mirabilis* liquefied the whole tube in 2 to 3 days. Proteus III did not liquefy when first tested, but after several transfers through broth produced a few liquefying colonies and subsequently liquefied regularly, though slowly, the change beginning at 10 days.

Variation or loss of proteolytic capacity and lack of correlation between different proteolytic reactions is well known. Thus, Wenner and Rettger (1919) found 4 strains of proteus out of 73 which did

not digest milk, while 3 had lost the power of liquefying gelatin. Smith, Herter and Ten Broek (cited by Wenner and Rettger) also found differences in gelatin liquefaction and loss of liquefying power among proteus bacilli.

Odor. The finding of a putrid odor quite similar to that of ozaena cases is common in these cultures, especially if they are tightly stoppered. It is strongest in infusion broth, agar plates, and gelatin, least in the corresponding meat extract media, and quite distinct in the egg-cube medium. In Group I the odor is moderate in the old Hofer Perez strain but strong in the others; strong in all strains of Group II; while in Group III it is moderate in Proteus II, but weak in Proteus I, III, IV and V and *Proteus mirabilis*. The difference is always one of degree only, varying in general with the age of laboratory cultivation, and not in the distinctive character of the peculiar fetor.

The differential fermenting properties are of special interest. The following reactions, as observed in Barsikoff's medium are summarized in Table I. Acid formation, as well as coagulation and precipitation of the nutrose, is noted.

Glycerol. Group I ferments; Group II, all ferment, while Strains I and II also coagulate and precipitate. In Group III all strains ferment except *Proteus mirabilis*. All except Proteus IV precipitate and coagulate.

Maltose. Group I ferments; Group II turns litmus red but reverts to blue in 10 to 20 days; Group III ferments. Proteus IV and *Proteus mirabilis* maintain the acid reaction, while Proteus I, II, III, and V revert to blue in 10 days.

Horowith (1916) found one proteus strain out of 24 which did not ferment maltose, while Wenner and Rettger (1919) obtained acid and gas with only 23 out of their 73 proteus cultures.

Dextrose. All strains ferment. Only Proteus III coagulates and precipitates.

Galactose. Group I ferments on the sixth day; Group II ferments, with coagulation and precipitation; Group III ferments. Proteus I, II, and III also coagulate and precipitate.

Saccharose. Group I does not ferment. Group II ferments in 4 to 10 days; Group III ferments, except *Proteus mirabilis*.

Xylose. Group I shows a slight reddening on the second or third day, but on the fourth or fifth day reverts to blue. Group II and Group III ferment.

Discussion

In the above observations the chief differential characters are seen to lie in the motility and in the cultural reactions in lead acetate agar, milk, gelatin, and the fermentation of saccharose and xylose. Group I is not motile, ferments maltose, but not xylose or saccharose. Groups II and III show more or less active motility, ferment xylose and saccharose, and, although they ferment maltose, revert to alkali (except in the case of two strains). Most of the motile strains coagulate milk and liquefy gelatin. The fermentation of maltose, though with reversion in the case of most of the motile strains, is a point of similarity between them and the non-motile group. Another is the failure on the part of some of the motile strains to liquefy gelatin or coagulate milk. An exception in this grouping is *Proteus mirabilis*, a motile organism which fails to ferment glycerol or saccharose. It is well known that Proteus may lose fermentative properties, and therefore differences in fermentation reactions may be no indication of essential differences between bacilli of this large group. Motility, on the other hand, is a more constant characteristic of bacteria in general. Nevertheless, Braun, and Schaeffer (1919) reported that Proteus X^{19}, grown on phenol agar, became non-motile and lost its flagella. Bornstein (1920) described the variety O Proteus of Weil Felix as a non-spreading, non-motile strain. Perez (1899) and Shiga (1922, 1923) describe their organism as non-motile or motile to a varying degree and identify them as the same bacillus. We cannot accept this conclusion. May we not, rather, suppose that in the Proteus group there are motile and non-motile strains, as in the B. coli group? We may then consider the type Perez bacilli as closely similar to B. proteus, a sub-group or sub-species of the large group of proteus bacilli, and Shiga's *B. ozaenae liquefaciens* strains as morphologically and culturally identical with *B. proteus vulgaris*.

AGGLUTINATION

We made 4 different agglutinating serums, 3 with Perez strains (Perez Hofer, Perez II, and Perez X) and one with Strain I of Group II. We injected rabbits, first with a heated saline emulsion of a 24-hour agar slant culture subcutaneously, then with a living emulsion subcutaneously, and finally, intravenously, using as much as 2.0 cc. of a 10.0 cc. saline emulsion. Some of the rabbits showed one or more abscesses at the site of subcutaneous inoculation and all became very weak. Eight days after the last injection they were bled.

All 14 strains were tested against the 4 serums (Table II). Serum, Perez Hofer and serum Perez II agglutinated only Perez Hofer, Perez II and Perez XI. (When first isolated, Perez XI was agglutinated only by serum Perez X, but after several transfers, also by the other three serums.) Serum Perez X agglutinated Perez X and XI and Strains I, II, III, and IV in the same dilution, but Proteus II in a higher dilution (1–6000). Serum Strain I agglutinated Perez Hofer, Perez II, and Perez XI, but all the strains of Group II, as well as Proteus II, in higher dilutions.

Such cross agglutinations would seem to indicate a close relation between the strains. However, attempts in the past to utilize the heterogeneous agglutinating properties of the Proteus group for purposes of classification have been fruitless. Horowith, Wenner and Rettger, Bornstein and others failed to find agglutinability among strains morphologically and culturally typical. Shiga had the same experience with his liquefying strains. It is also known that anti-proteus serum has group and collateral agglutinating properties Dopter and Sacqueppé found agglutinating power for *B. coli communis* and *B. typhosus* in such serums. We may suppose that agglutination is probably a non-specific colloidal reaction. It would follow that agglutinability by an anti-proteus serum indicates close similarity in colloidal structure rather than identity on the part of a given bacillus. To analyze this structure we did agglutinin absorption tests. For convenience we term the various specific group or collateral agglutinating powers the "branches" of an agglutinating serum.

Sedimented bacteria, washed in saline from 24-hour agar slants,

TABLE II

Agglutination of all strains with four immune serums

	DILUTIONS	SERUM PEREZ HOFER									SERUM PEREZ II								
		1/100	1/200	1/500	1/1000	1/2000	1/4000	1/6000	1/8000	1/10,000	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/6000	1/8000	1/10,000
Group I	Perez Hofer	+++	+++	+++	+++	+++	+	–	–	–	+++	+++	+++	++	++	–	–	–	–
	Perez II	+++	+++	+++	++++	++	+	–	–	–	+++	+++	+++	+++	++	++	–	–	–
	Perez X	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Perez XI	++	+	–	–	–	–	–	–	–	+++	++	+	–	–	–	–	–	–
Group II	Strain I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Strain II	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Strain III	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Strain IV	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Group III	Proteus I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus II	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus III	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus IV	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus V	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus mirabilis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

	DILUTIONS	SERUM PEREZ X									SERUM STRAIN I								
		1/100	1/200	1/500	1/1000	1/2000	1/4000	1/6000	1/8000	1/10,000	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/6000	1/8000	1/10,000
Group I	Perez Hofer	–	–	–	–	–	–	–	–	–	+++	+++	+++	++	–	–	–	–	–
	Perez II	–	–	–	–	–	–	–	–	–	+++	+++	+++	+++	++	–	–	–	–
	Perez X	+++	+++	+++	+++	++	–	–	–	–	–	–	–	–	–	–	–	–	–
	Perez XI	+++	+++	+++	++	+	–	–	–	–	+++	++	+	–	–	–	–	–	–
Group II	Strain I	+++	+++	+++	+++	++	–	–	–	–	+++	+++	+++	+++	+++	+++	+++	+++	++
	Strain II	+++	+++	+++	++	+	–	–	–	–	+++	+++	+++	+++	+++	+++	+++	+++	++
	Strain III	+++	+++	+++	++	+	–	–	–	–	+++	+++	+++	+++	+++	+++	+++	++	+
	Strain IV	+++	+++	+++	+++	++	–	–	–	–	+++	+++	+++	+++	+++	+++	+++	+++	++
Group III	Proteus I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus II	+++	+++	+++	+++	+++	++	+			+++	+++	+++	+++	+++	+++	+++	+++	++
	Proteus III	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus IV	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus V	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Proteus mirabilis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

were added to the immune serum, diluted 1 to 100, at intervals of 2 hours, until no further flocculation occurred. The serum with bacteria was then placed in the ice-box overnight and centrifugalized the next morning. Sometimes there was a slight opalescence, but this never disturbed the sharpness of subsequent reactions. The dose necessary for complete absorption varied. For serums Perez Hofer and Perez II, one slant of the corresponding culture, respectively, was sufficient to remove their three branches. For serum Perez X the branches to strains Perez X and XI required three slants,

TABLE III

Agglutination after agglutinin absorption. Serum Perez Hofer

	PEREZ HOFER	PEREZ II	PEREZ XI
Unabsorbed	1/2000	1/2000	1/500
Absorbed with Perez Hofer	–	–	1/500
Absorbed with Perez II	>1/1000	–	–
Absorbed with Strain 1	>1/1000	>1/000	–

TABLE IV

Agglutination after agglutinin absorption. Serum Perez II

	PEREZ HOFER	PEREZ II	PEREZ XI
Unabsorbed	1/2000	1/4000	1/1000
Absorbed with Perez II	1/500	–	1/500
Absorbed with Perez Hofer	–	1/1000	–

that to Strain II required one slant, and those to Strains II, III, IV and Proteus II required two slants of the respective cultures, and this in spite of the equal degree of agglutination of all these strains by this serum. For serum Strain I, $3\frac{1}{2}$ slants were required to absorb the branch for Strain I; for branches to Perez Hofer, Perez II, Strains II and III, one slant, and for Proteus II, two slants.

The results of these tests are summarized in Tables III, IV, V, and VI. The absorption of the principal branch does not necessarily remove all other branches, a finding contrary to the principle of Castellani. The closest approximation to complete removal of all sec-

TABLE V

Agglutination after agglutinin absorption. Serum Perez X

	PEREZ X	PEREZ XI	STRAIN I	STRAIN II	STRAIN III	STRAIN IV	PROTEUS II
Unabsorbed	1/2000	1/2000	1/1000	1/2000	1/2000	1/2000	1/6000
Absorbed with Perez X	–	–	1/1000	>1/1000	>1/1000	1/1000	>1/1000
Absorbed with Perez XI	–	–	1/1000	>1/1000	>1/1000	>1/1000	–
Absorbed with Strain I	>1/1000	>1/1000	–	–	–	–	–
Absorbed with Strain II	>1/1000	1/500	–	–	–	–	–
Absorbed with Strain III	>1/1000	>1/1000	1/100	–	–	–	–
Absorbed with Strain IV	1/100±	–	1/100	–	–	–	–
Absorbed with Proteus II	>1/1000	>1/1000	>1/1000	1/100	–	–	–

TABLE VI

Agglutination after agglutinin absorption. Serum Strain I

	PEREZ HOFER	PEREZ II	PEREZ XI	STRAIN I	STRAIN II	STRAIN III	STRAIN IV	PROTEUS II
Unabsorbed	1/1000	1/2000	1/500	1/10,000	1/10,000	1/10,000	1/10,000	1/10,000
Absorbed with Perez Hofer	–	–	–	>1/1000	>1/1000	1/500	1/1000	>1/1000
Absorbed with Perez II	1/500	–	–	>1/1000	>1/1000	>1/1000	>1/1000	>1/1000
Absorbed with Perez XI	1/1000	–	–	>1/1000	>1/1000	>1/1000	>1/1000	>1/1000
Absorbed with Strain I	–	–	–	–	1/1000	–	–	–
Absorbed with Strain II	–	1/500	1/100	1/500	–	>1/1000	–	>1/1000
Absorbed with Strain III	1/500	–	–	>1/1000	>1/1000	–	–	>1/1000
Absorbed with Strain IV	1/1000	–	–	>1/1000	>1/1000	–	–	–
Absorbed with Proteus II	1/500	1/100	–	>1/1000	>1/1000	–	–	–

ondary branches was in the case of serum Strain I, absorbed with Strain I, which lost all branches except that for Strain II which was reduced. If a branch for one of the non-motile strains is absorbed out, most but not all of the branches for non-motile bacilli are removed or reduced, while those for motile bacilli are unaffected. One exception was found: serum Perez X, absorbed with Perez XI, lost the branch for Proteus II. Similarly, if one motile branch is absorbed out, all other motile branches are removed, while the non-motile branches are unaffected. If serum Perez X has one of the motile branches absorbed out, all motile branches are removed or reduced, while most of the non-motile branches are unaffected. With serum Strain I the results are more irregular, but the general principle obtains.

We found that if, in a given case, cross saturation shows identical group agglutinative branches, this is no proof of the identical nature of the bacilli. For instance, if we absorb serum Strain I with Perez Hofer, the branches for Strain III and Strain IV are merely reduced. But if we absorb serum Strain I with Strain III the branch for Perez Hofer, is reduced; and if we absorb with Strain IV the branches for Perez Hofer, Strains I and II and Proteus II are unaffected, but Perez II and XI are removed. If we absorb serum Strain I with Perez II or XI, the branches for Perez Hofer, for Strains I, II, III and IV, and for Proteus II are unaffected. If serum Perez X is absorbed with Strain IV, all motile and non-motile branches are removed except that for Strain I, which is reduced. If serum Perez Hofer is absorbed with Strain I, the branches for Perez Hofer and Perez II are unaffected, while that for Perez XI is removed. But if serum Perez X is absorbed with Strain I, the branches for Perez X and XI are unaffected.

Although some of these bacilli give identical agglutinative reactions in certain cases, we are still unable to conclude that they are identical. Perez Hofer is not identical with Strain III or Strain IV; Strain IV is not identical with Perez X; Strain I is not identical with Perez XI. It is necessary to absorb all branches of each serum, and then it becomes evident (1) that each branch has individuality and (2) that the constitution of Perez bacilli is very close and similar to that of Proteus.

COMPLEMENT FIXATION

Calmette demonstrated that the degree of adaptation between antigen and immune serum determines the amount of complement which will be absorbed. In our work we wished to study the relationship of each strain, as antigen, to each serum. We took constant doses of bacillary antigen and of serum, carefully titrated before use. Antigens were prepared by emulsifying 24-hour agar slant cultures in 10 cc. of saline and heating for one-half hour at 60°C. The complement and hemolytic amboceptor were carefully titrated as usual. For each antigen we set up a series of four tubes with two units of complement in the first and two additional units in each succeeding tube. There was a control tube for each amount of complement and two antigen control tubes in single and double quantities (Table IV).

We found that the three groups have an identical antigenic property. Perez Hofer and Perez II, Strain I and Proteus II react similarly to serum Perez Hofer. Perez Hofer antigen shows no fixation with serum Perez X and absorbs only two units of complement with serum Strain I. Antigen Strain I absorbs only four units with serum Perez II, but more than eight units with serum Perez Hofer and serum Strain I. Antigen Proteus II absorbs more than eight units with serum Perez Hofer and serum Strain I, but only four units with serum Perez X. The results are summarized in Table VI.

Complement fixation, like agglutination, is a non-specific reaction, but it shows more distinctly than the latter the relationship and similarity of the colloidal structure of different antigens in relation to the serums. We did not find a parallelism between agglutination and complement fixation. As in the finding of Dopter and Sacqueppé regarding proteus agglutinins, we found in our control tests that serum Perez Hofer gives a slight fixation with *B. coli* antigen; that serum Perez II also gives a fixation with *B. coli* and *B. typhosus*, but that serums Perez X and Strain I do not fix complement with "*coli*" or "*typhosus*" antigen. None of the four serums fix complement with *B. pyocyaneus* antigen. The difference between *B. coli* or *B. typhosus* and the proteus group shows that complement fixation furnishes no proof of identity of different bacilli and only indicates their close similarity to each other.

TABLE VII

Titration of complement fixed by four immune serums with various antigens

Method of setting up tubes

	TUBE 1	TUBE 2	TUBE 3	TUBE 4	TUBE 5	TUBE 6	TUBE 7	TUBE 8	TUBE 9	TUBE 10
Antigen	0.3	0.3	0.3	0.3	0	0	0	0	0.3	0.6
Immune serum	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0
Complement	0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8	0.2	0.2
Sensitized cells	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Saline	1.3	1.1	0.9	0.7	1.6	1.4	1.2	1.0	1.5	1.2

Results with serum Perez Hofer

Antigen	TUBE 1	TUBE 2	TUBE 3	TUBE 4	TUBE 5	TUBE 6	TUBE 7	TUBE 8	TUBE 9	TUBE 10	UNITS OF COMPLEMENT FIXED
Perez Hofer	−	−	−	−	+	+	+	+	+	±	8+
Perez II	−	−	−	−	+	+	+	+	+	±	8+
Strain I	−	−	−	−	+	+	+	+	+	±	8+
Proteus II	−	−	−	−	+	+	+	+	+	±	8+
Pyocyaneus	+	+	+	+	+	+	+	+	+	−	0

Results with serum Perez II

Perez Hofer	−	−	−	−	+	+	+	+	+	±	8+
Perez II	−	−	−	−	+	+	+	+	+	−	8+
Strain I	−	−	−	−	−	±	+	+	+	−	4+
Strain II	−	−	−	−	+	+	+	+	+	±	8+
Pyocyaneus	+	+	+	+	+	+	+	+	+	−	0

Results with serum Perez X

Perez X	−	−	−	−	±	+	+	+	+	−	6+
Strain I	−	−	−	−	±	+	+	+	+	−	6+
Proteus II	−	−	−	±	−	+	+	+	+	−	4
Perez Hofer	+	+	+	+	+	+	+	+	+	±	0
Pyocyaneus	+	+	+	+	+	+	+	+	+	−	0

Results with serum Strain I

Strain I	−	−	−	−	+	+	+	+	+	±	8+
Perez Hofer	−	±	+	+	+	+	+	+	+	±	2
Perez X	±	+	+	+	±	+	+	+	+	±	0
Proteus II	−	−	−	−	+	+	+	+	+	−	8+
Pyocyaneus	+	+	+	+	+	+	+	+	+	−	0

FLOCCULATION

We did only a few flocculation experiments for lack of sufficient serum. Flocculation occurred at room temperature in half an hour with 0.5 cc. of immune serum and 2 or 3 cc. of toxin. Toxin Perez Hofer produced coarse flocculation with serums Perez Hofer and Perez II, but none with serum Strain I. Toxin Perez II produced flocculation with serums Perez II and Perez Hofer, but not with serum Strain I. Toxin Strain II produced coarse flocculation with serums Strain II and Perez X, but not with serum Perez Hofer. Toxin Strain III produced flocculation with serum Strain I and slight flocculation with serum Perez Hofer. The flocculation test, like that of agglutination and complement fixation, shows the close relationship between the typical Perez bacilli and *B. proteus*, but not their identity, for each strain has some individual peculiarity.

PATHOGENICITY

Perez, Shiga and others thought that typical ozaena bacilli, motile or liquefying (*B. ozaenae liquefaciens*, Shiga), are more pathogenic for rabbits than for other animals. All our strains have the same pathogenic effect. Most of them are pathogenic only for the rabbit, whether injected subcutaneously, intraperitoneally, or intravenously. Guinea-pigs and rats are susceptible to a few of our strains. These strains, injected subcutaneously, cause abscess formation or death, but, intraperitoneally, kill all animals with a purulent peritonitis. The susceptibility of the rabbit varies. Young rabbits are more susceptible, some large ones not at all. Intracutaneous injection produces a large erysipelatous inflammation. After several days the skin becomes ulcerated, necrotic, and brown, discharging a purulent exudate. It heals very slowly, in some cases not for two months. Subcutaneous injection produces a very different effect. A large dose produces death in one to several days. If death occurs in 24 to 48 hours, the nose and thymus are congested and hemorrhagic. Twice we found a large external hemorrhage from the nose, covering the hairs of the chest. The lungs are congested and in some cases we found an acute hemorrhagic peritonitis or pericarditis. If death occurs after 20 to 30 days, the rabbit has become very emaciated.

At autopsy there is a large abscess at the site of the inoculation, full of thick white pus; the nearest lymph glands are suppurating, and sometimes there are innumerable small proliferative peritoneal lesions, in the shape of white, pedunculated or flat nodules on the peritoneum. Some appear greasy, while others, on section, are found to be filled with thick white pus. The spleen is sometimes congested, likewise the liver. In some cases the liver presents miliary focal abscesses. If the dose injected is not sufficient to produce early death, or if the susceptibility of the rabbit is slight, a large subcutaneous abscess is formed. After several days the abscess ruptures and white pus is discharged. The discharge persists for a long time, the skin becomes brown and necrotic, and heals after several months.

Intraperitoneal injection is more injurious, producing hemorrhagic peritonitis if death occurs in one or two days, or purulent peritonitis in several days. Then all the characteristic lesions of the thymus, nose, lungs, etc., are found.

The intravenous injection is the most injurious. With a sufficient dose, death occurs in 12 to 24 hours. The blood is full of bacilli of the same type. The nose is congested and often external hemorrhage occurs. The thymus is contested and full of infarcts, and sometimes it is very large, covering the heart and the edges of the lungs. The lungs are congested and hemorrhagic pericarditis or peritonitis may be present.

A broth culture of 48 to 72 hours is more pathogenic than one of 24 hours. We agree with Shiga that death is due more to the toxicity of the bacilli than to the septicemia, although we never succeeded in finding a free toxin (exotoxin) in broth cultures in less than 4 days' incubation.

If the injected dose does not produce death in a few days the rabbits recover, or succumb later with characteristic lesions. The following protocols are typical.

Rabbit 4. Inoculated January 26 with 0.5 cc. of a 24-hour infusion broth culture of Strain II (Group II) subcutaneously. February 7, did not show any general pathogenic effect. Site of inoculation swollen. February 7, inoculated with 0.25 cc. of a 24-hour infusion broth culture of Strain II intravenously. On the sixth day (February 13) there was a slight mucopurulent discharge from the

nose. A culture made from this discharge showed the original bacillus, Strain II, in pure culture. The rabbit was very weak and emaciated. March 12 there was a definite paralysis of the posterior limbs. Both eyelids were congested, with a dry necrosis on the edges. Death March 15, 48 days after inoculation. The nose was filled with a mucopurulent material. The nasal mucosa was congested throughout and mottled, being a deep purplish red in some areas and lighter, and somewhat opaque, in others. The edges of the turbinate bones were irregular, showing a few superficial crateriform ulcers. The thymus was normal, as were

FIG. 1. RABBIT 15, SHOWING SYMMETRICAL NECROSIS OF EARS. INJECTED INTRAVENOUSLY WITH STRAIN III (B. PROTEUS)

all the other organs. Cultures made from the nasal discharge did not yield Strain II. Culture from the heart's blood was sterile.

Rabbit 15. Inoculated intravenously February 26 with 0.5 cc. of a 24-hour infusion broth culture of Strain III (Group II). On March 22 the rabbit still showed no pathogenic effect. Toward the end of the month it became emaciated and a paralysis of the posterior limbs set in. The ears showed a symmetrical necrosis, more extensive on the right (Fig. 1). The rabbit was killed April 7. *Autopsy:* Nasal congestion and slight mucopurulent secretion. Thymus normal. Other organs normal. Culture from the heart's blood sterile. Strain III not found in culture of mucopurulent secretion from the nose.

As Perez, Shiga and others showed, it is not always easy to produce in every case a symmetrical necrosis or mucopurulent nasal secretion. Often when we injected small doses intravenously, the rabbits did

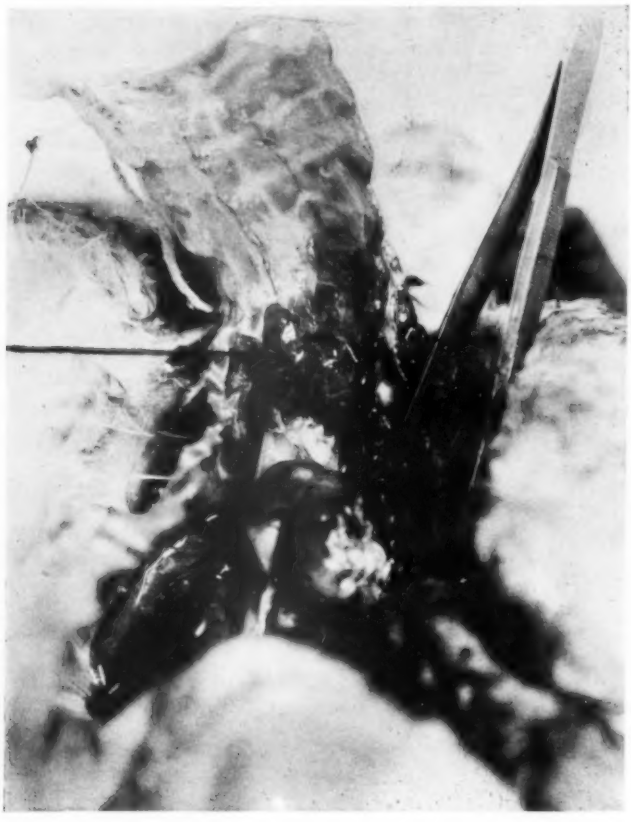

FIG. 2. HEMORRHAGIC INFARCTS IN THYMUS

not show any lesions, or, after a month or more, developed a distinct paralysis which subsequently disappeared. One rabbit showed a characteristic paralysis on the third day after inoculation with Hofer's Perez bacillus, but at death, two days later, did not present anything

but the usual acute congestion and hemorrhagic, toxemic, and septicemic lesions.

The necrotic lesions are very rare. They have been described by others as usually dry. In 70 rabbits inoculated with different doses and strains, we found only 2 with characteristic necrotic symmetrical lesions. The necrosis was produced not only in the ears and nose, but on the eyelids. It was dry, and seemed to begin around the capillaries. Sections showed endarteritis and periarteritis of the arterioles. As Shiga found, with his liquefying and also with his non-liquefying strains, so we found that the Perez or Proteus bacilli have an affinity for the blood vascular system, producing at first stasis and extreme congestion, so that the capillaries may rupture with interstitial or external hemorrhage. In the second stage dry necrosis occurs following capillary injury. This last lesion is rare, because death usually results from the toxemia and septicemia before necroses have an opportunity to develop.

The congestive and hemorrhagic lesions produced by Perez or Proteus bacilli are not exclusively characteristic for these groups; many other bacilli are known to cause such lesions. We injected intravenously 2 cc. of an infusion broth culture of two different strains of *B. coli*. After 24 hours the two rabbits were dead and the autopsy showed congestion of the nose and lungs and the thymus congested and turgid with blood. The congestion of the thymus, however, was less, without infarcts and without exudate or hemorrhage into the serous cavities.

Discussion

Perez, Shiga, and others described symmetrical necrotic lesions produced only by the typical Perez bacillus. Shiga, with his *B. ozaenae liquefaciens*, produced similar lesions. Our strains II and III, identified as typical Proteus bacilli, produce the same picture. The suppurative and congestive lesions are produced with the most Typical, Proteus I and II. Larson and Bell, studying the pathogenic property of *B. proteus*, found the exudative and proliferative lesions and described the toxemic-septicemic picture without mentioning the congestion of the nose and thymus. They did not try to produce a toxin. Shiga, studying his *B. ozaenae liquefaciens*, identical with

our Group II, which we identified as typical Proteus, was the first to find and describe the congestion of the nose and thymus. We reproduced the same lesions with Proteus I and II as with Hofer's Perez bacillus.

TOXICITY

All of our isolations, as well as Hofer's Perez bacillus and Proteus I, produce the same toxin as described by Shiga. In infusion broth at 37°C. the toxin appears after 4 to 5 days, is strongest at 6 to 7 days, and disappears at 9 to 10 days. The toxin has the property of a *B. proteus* toxin, as described by many authors. It is not very strong, 1 to 2 cc. being necessary to produce death; is very labile, disappearing at room temperature in 24 to 48 hours, and in the ice-box in about 2 to 3 days. We tried by various devices to produce a stronger toxin. The strongest toxin was found in infusion broth with rabbits' red cells or whole blood. The addition of serum had no effect. After 6, 8, and 10 days the blood broth was filtered through a Berkefeld candle. Half a cubic centimeter of a filtrate of a 6- and 8-day culture kills rabbits regularly in 18 to 24 hours, while that of a 10-day culture is not toxic. At autopsy there are the same acute congestion and hemorrhagic lesions as are produced by a living broth culture of Perez or Proteus; nasal congestion and sometimes internal or external hemorrhage; thymus congested and infarcted; lungs congested; and sometimes hemorrhagic pericarditis or peritonitis. We injected small doses into rabbits but did not succeed in producing the symmetrical necrosis. One rabbit become lop-eared. The toxin is not harmful to guinea-pigs when injected intracardially or subcutaneously, whether of Hofer's Perez bacillus or of *B. proteus*. For the rat 2 cc. of an infusion broth filtrate, injected subcutaneously, is without effect, a dose which kills the rabbit in 18 to 24 hours. The toxin injected subcutaneously into the rabbit does not produce necrosis.

Our strains of Perez bacilli, as well as proteus, have the same toxigenic property and produce the identical toxin. We tested the toxigenicity of Proteus I and II, very old laboratory cultures. Proteus I became toxigenic after two passages through guinea-pigs, while Proteus II, isolated two years before and pathogenic, was not toxi-

genic. Our strain Perez II bacillus was at first toxigenic, but lost this property after a year, and when tested on two occasions showed no toxin. All our strains of Group I and Group II produce a toxin identical with that of a typical Proteus I.

Discussion

The toxicity of the Proteus group has been described and studied since the work of Hauser. According to Gouget, Hauser, Hofmeister, Lannelongue, and Achard, the proteus bacillus kills animals only through toxicity and not by multiplication and septicopyemia. Foa and Bonome, Schlintzler, Krozius, and Wredon hold the opposite view. The identity of anatomico-pathological lesions, found after the injection either of a toxin or of a living broth culture, proves that death is due to the toxicity, possibly from the presence of Zinsser's X substance. Older broth cultures produce death more rapidly and with smaller amounts and when death occurs in one or several days, before the formation of an abscess, it is due to the toxicity, as Shiga believes. The toxin has an affinity for the blood-vessels. The capillaries are congested; some rupture, producing infarcts or external hemorrhages. This congestion and the ruptures were described by Gouget in his study of the pathogenic rôle of Proteus in hepatic infections. Many others have described the congestive effects of proteus toxin. Shiga found the same for his *B. ozaenae liquefaciens* strains, as well as for typical Perez. Our most typical strain, Proteus I, produced the identical toxin, as did all our strains of Group I and Group II. Before Shiga, none mentioned the congestion and hemorrhage into the thymus. With the toxin of Hofer's Perez bacillus, as well as with Perez X and XI, with the liquefying Strains I, II, III and IV, and with Proteus I, we produced regularly the congestion, infarction, and cloudy swelling of the thymus, lungs, and nose, and very often an external hemorrhage of the nose and hemorrhagic peritoneal or pericardial exudate. Like Shiga, we did not succeed in producing an antitoxin with any of our strains.

RECAPITULATION OF THE PATHOGENIC ACTION OF THE PROTEUS GROUP

Many bacilli of this group are not pathogenic for rabbits or other animals. Those pathogenic for rabbits may be non-pathogenic for the rat or guinea-pig. The pathogenic action for rabbits is (1) toxico-septicemic with acute congestive hemorrhagic lesions. The toxin and the fresh broth culture produce the same anatomic-pathologic lesions, congestion, vascular rupture, and hemorrhage. The guinea-pig and rat never presented this picture, either after infection or injection of toxin. (2) Chronic pyemic. The bacilli injected intravenously, intraperitoneally, or subcutaneously in sufficient amounts (provided death dose not occur at once and the animal has time to react) are disseminated throughout the organs, earliest and in greatest numbers in the mucous membranes, peritoneum, pericardium, and nasal mucosa, producing exudative and proliferative lesions, abscesses in the lymph glands and spleen, and sometimes in the liver. In some cases, when death does not occur early, mucopurulent nasal discharge and superficial ulceration over the turbinate bones may develop. The guinea-pig and rat may show this pathological picture. (3) Local. If sufficiently small amounts of bacilli are injected intraperitoneally, subcutaneously or intracutaneously, the infection may be localized and the animal survives. When pus is formed, *B. proteus* cannot always be isolated, and very often the congestive hemorrhagic lesions disappear before the death or the sacrifice of the animal. The exudative lesions heal slowly and secondary infection may occur. (4) Peculiar dry necrotic lesions, as are sometimes found after intracutaneous injections.

CROSS IMMUNIZATION

The 4 rabbits immunized for agglutinin were tested for cross immunization. Rabbit I, immunized with Hofer's Perez bacillus, was inoculated intravenously with 4 lethal doses (2 cc.) of a 24-hour infusion broth culture of *Proteus vulgaris* (Proteus I) on April 20th. The control animal died within 24 hours, while rabbit I was alive and in good condition more than a month later.

Rabbit II, immunized with Perez II, was injected intravenously

April 20th with 4 lethal doses (4 cc.) of a 24-hour infusion broth culture of Proteus II. The control animal died within 24 hours, while rabbit II was still in good condition 13 or 14 days later. Then it became ill, grew weak, and on May 10th it died, 20 days after inoculation. At autopsy the thymus and nose were normal, the peritoneal and mediastinal lymph glands caseous. Subcutaneous abscess was present, not ruptured, filled with grayish white thick pus. Other organs macroscopically normal. Culture of heart's blood sterile. Culture of caseous lymph gland gave pneumococcus, *Micrococcus catarrhalis*, and Proteus II.

Rabbit III, immunized with Perez X, was inoculated intracardially April 20th with 4 lethal doses (4 cc.) of Strain I (Group II, *Proteus vulgaris* type). Control animal dead within 24 hours. Rabbit III dead after 20 days. Autopsy: Thymus and nose normal. Pericardial sac enormously distended with very thick white pus. Innumerable subperitoneal abscesses from 2 to 15 mm. in diameter, some flat but the majority pedunculated. All these nodules were white, lardaceous, and on incision were seen to be filled with very dry inspissated pus. Culture gave staphylococci and *B. coli*. Peritoneal abscess and pericardial pus, sterile. Culture of heart's blood gave staphylococci and *B. coli*.

Rabbit IV, immunized with Strain I (Group II, Proteus vulgaris type). The rabbit became very weak and emaciated, and we did not test it for cross immunization at the same time as the other three. It was inoculated April 14th with 4 lethal doses of Hofer's Perez bacillus. Death April 25th, at the same time as the control. Autopsy: Lesions of chronic proteus infection. Peritonitis with abscesses from 2 to 15 mm. in diameter, identical with those of Rabbit III. A few mesenteric glands were caseous. The peritoneal surface of the liver was densely seeded with subperitoneal and subcapsular miliary abscesses. Similar abscesses were also found densely scattered throughout the hepatic substance. The kidney showed the same dense infiltration with miliary abscesses over the peritoneal surface, but the substance of the organ showed only a few scattered foci. Spleen congested. Adhesions between intestines, liver, and diaphragm, although the rabbit had never been injected intraperitoneally. Nose, thymus, and lungs normal. Caseous mediastinal

glands. Blood culture gave Perez Hofer and *B. coli*. Culture of pus negative.

Discussion

The cross immunizations show that typical Perez bacilli may immunize against typical *B. proteus*. The rabbit immunized with Perez Hofer is completely protected against Proteus I. Rabbits II and III, also immunized with Perez bacillus, acquired some resistance against Proteus, while Rabbit IV, which was suffering from chronic pyemic proteus infection, may be assumed to be entirely unprotected against any other strain of Proteus. This is to be expected, since the Proteus group is very large and, as our serological findings show, strains which are culturally and morphologically identical may have different serological properties.

SUMMARY AND CONCLUSIONS

1. Although the cultural and fermentative properties are insufficient to identify Perez bacillus as atypical Proteus, we are justified in thinking that it constitutes a sub-group which has lost some of its fermentative properties, a frequent occurrence with the most typical proteus, and that *B. ozaenae liquefaciens* Shiga is a typical *B. proteus vulgaris*.

2. The agglutination and agglutinin absorption tests show Perez bacillus to be very closely related to *B. proteus;* Perez X is intermediate between the two species, having agglutinative properties as strong for Perez bacillus as for *B. proteus*. Each bacillus of this group has a serological individuality more or less close to the Perez or proteus species. Of the 14 bacilli, no two are identical. Agglutinin absorption shows clearly that the motile strains, *B. ozaenae liquefaciens* Shiga, and *B. proteus vulgaris*, as a rule, have their co-agglutinative branches reduced or removed when absorption is done with the motile bacillus, and vice versa; they are more closely related to each other than to the non-motile Perez species and can be identified as belonging to the same group as *B. proteus*.

3. Complement fixation shows that the two varieties, motile and non-motile, have a very close relation. Some Perez and Proteus bacilli have identical properties as antigens against a given serum and this

reaction appears to show closer relationship than the agglutination or agglutinin absorption test. There is no correlation between the complement fixation and agglutination test.

4. The more specific flocculation reaction also shows the organisms to be very closely related, but only Perez II and Hofer's Perez bacilli have *identical* properties, while of these only the latter shows flocculation with *B. proteus*.

5. *B. proteus*, *B. Perez*, and *B. ozaenae liquefaciens* have identical pathological properties. Each produces the four different types of infection: (a) toxico-septicemic congestive and hemorrhagic lesions; (b) chronic pyemic, mucosal, and endothelial exudative and proliferative lesions; (c) local exudative lesions; and (d) special necrotic lesions due to complex pathological processes.

6. All our strains produce a toxin identical with that of Hofer's Perez bacillus and *Proteus vulgaris*.

7. Cross immunization shows that Perez bacillus and *B. proteus* have identical immunizing properties, protecting completely or producing sufficient protection to indicate their identity or very close relationship.

On the basis of the above conclusions it follows that the Perez bacillus is a member of the large group of Proteus bacilli.

8. The etiological relation of the Proteus-Perez group to ozaena foetida has been suggested by many workers because of the frequent finding of these organisms in the nasal discharge of such cases. The Proteus group exhibits an affinity for the blood vessels and mucosae, has pathogenic power, producing chronic necrotic lesions and nasal ulceration and discharge. We never produced green crusts or atrophy of the turbinate bones in the rabbit, and therefore we cannot conclude that in human beings *B. proteus* produces ozaena foetida, and that this bacillus acts as the primary etiological agent. The frequency of its occurrence and the specific malodor found in the cultures show that *B. proteus* is involved in the pathogenesis of ozaena, and whether implanted primarily or secondarily, is the cause of the fetor, discharge, and ulceration.

It is a pleasure to acknowledge our indebtedness to Dr. W. W. Ford and to Dr. S. J. Crowe, for according us the necessary facilities and for suggestions; and to members of their departments for various courtesies.

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BOOK REVIEWS

Thomas Sydenham, Clinician. By DAVID RIESMAN, M.D., Professor of Clinical Medicine, University of Pennsylvania. (*Paul B. Hoeber, Inc., New York, MCMXXVI.*)

Lieut.-Col. Fielding Garrison, in whose mature judgment all students of the History of Medicine have the greatest confidence, has often said that, here in America, we need today a series of short, compact books or "Primers," each devoted to some one subject in Medical History, written by a recognized authority, and without any attempt to lower the level of treatment to that of a mental "spoon-feeding," yet so compiled as to stimulate interest in the casual reader and to afford him, if he wishes it, some clear-cut suggestions and helps to further study. Dr. Riesman's Essay on Sydenham, written for the tercentenary of Sydenham's birth may be welcomed as a specimen of the type of book that Col. Garrison describes. It is pleasant reading; it contains just enough personal anecdote to make it humanly interesting, while, at the same time, it sketches the historical background of Sydenham's life, gives the more important known facts of his career, points out his attitude towards various types of disease, and, finally, intersperses the narrative with enough judicious quotations to make the reader anxious to read a little more of Sydenham himself. An anxiety of a most wholesome type. For today, in the haste of professional or academic life, we read, if we read at all, too much *about* our "Fathers of Old," and far too little the Fathers themselves.

In reading Sydenham, either in Latin or English, it is most interesting to check off the echoes from Hippocrates. It is true that he seldom cites Hippocrates directly, or more seldom still gives an exact reference. (In this, Dr. Riesman follows Sydenham's example, for he never gives a single reference for any of his quotations, so that one has to hunt through the "Opera Universa" in order to check them up. And one would be grateful, especially for the source of the Greek quotation from Hippocrates on page 37.) Nevertheless, one recognizes Hippocratic points of view, and even unconscious quotations, at every turn. In Dr. Riesman's Essay, the last paragraph on pages 24 and 25, the test by fire and water for tuberculous sputum, as well as other passages, either in the Essay itself or in the quotations given in it, are all distinctly Hippocratic, and can easily be paralleled by passages from the Corpus. Coan too, and removed "toto caelo" from Cnidian influence, is Sydenham's attitude towards his work. To him, the Practice of Medicine is not a "Science;" it is, as it was to Hippocrates, "an Art," not to be learned by any moron who chose to apply his mind to it for a given period of time, but only open to the "Born Physician" who could add to certain god-given gifts of temperament and character, patient observation, love of mankind, and disciplined methods of logical thought.

Interesting, also, is Sydenham's Psychiatry (pp. 33, 36, 42, etc.), his recognition of psychasthenic elements in gout, his treatment of depressive conditions and psychogenic disturbances of the emotional equilibrium. He had caught the fundamental concept of the Coan School; that the physician is not treating a disease, but a patient who shows certain pathological reactions, and that he or she is not to be considered as made up of a body and a mind, one separated from the other, but as a personality, as an integrated individual.

Sydenham's work appeared first in Latin, in 1685, the first edition in English is dated 1696. (Dr. Riesman, by the way, in his useful bibliography (p. 49), gives the first English edition as of 1697, while, on the preceding page, his facsimile of the title-page of the first edition is dated 1696.) Now Sydenham's Latin is unusually good Latin for a busy physician. No doubt, in his year at Oxford, he learned to write Ciceronian prose. But one is surprised to note how much better his Latin was than the Latin of some of his contemporaries, Richard Morton, for instance (1637–1698). Compare with Morton's choppy style in his Case Histories this sentence on page 142 of the Opera Medica of Sydenham (Geneva, 1726), from the "Febris Continua of 1673–75"; "Quamvis autem Stupor hic ad Comatis ascendens naturam, huic febri aliis symptomatibus frequentius superveniebat, quandoque tamen, at rarius, Phrenisis absque Stupore nonnullos invadebat."

One has often wondered whether a busy physician, whose time was wholly taken up with his practice, did not have some learned secretary, some needy University scholar, who could "do the Latin" for him. In other words, whether the true first edition of many medical works was not first written in English, and then, later on, done into Latin, instead of vice versa. But this is an unkind, and no doubt, an unwarranted suspicion.

It would be still more unkind to point out occasional slips in Dr. Riesman's interesting Essay. But (p. 30, note) Smintheus, when used of the God Apollo, does not mean "Rat-Destroyer." Hesychius, in his "Dictionary," connects it, as do many others, with a town in Troas. Or it may be associated with a Cretan word for mouse, according to the Scholiast on the Homeric passage in which the name occurs. Nor should one quote the Biblical passage from Samuel I, chap. vi, without being sure what is the actual meaning of the Hebrew word, which, in our King James' version, is rendered by "mice." Then, one cannot but feel a little uncertain about the translation of the three Greek words from Hippocrates on p. 37. The second substantive, in the plural, could scarcely mean "Nature."

These are unimportant details.

In connection with the "Methodus medendi morbos per accubitum junioris" (p. 37), which would be so sympathetic to at least one class of modern men and women, Dr. Riesman might have appositely quoted from the Bible again. He must have forgotten old King David, and his treatment "per accubitum junioris by Abishag.

"Thomas Sydenham," says Dr. Riesman (p. 11), "was not regarded as a great man in his own time." He did not take "his degree of Doctor" (p. 15) till he was fifty-two years old. He was never elected to Fellowship in the Royal College of Physicians (p. 13). Yet posterity has forgotten even the names of the colleagues who "lorded it over him."

What a comforting thought in our hours of depression and discouragement. And stimulating, if it encourages us to follow his example, and "take a degree," or prove ourselves still mentally young, at fifty-two.

J. R. O.

A Text-Book of General Bacteriology. By EDWIN O. JORDAN. 752 pp. $5.00. (*W. B. Saunders Co., Phila., 1924.*)

Throughout its eight editions this has seemed to the reviewer to be the best written and best arranged of all English texts on this subject. It is readable and the selection of material reflects good judgment. However, in common with most other textbooks which pass through many revisions, there is a tendency for the revisions not to be thorough enough, so that one finds references to recent work incomplete. Methods for the study of anaërobic bacteria are not described as completely as might be desired. The Morax-Axenfeld diplobacillus is described as pathogenic only for the human eye, no reference being given to the work of F. S. Jones on a similar, if not identical, infection in cattle. The classical work of Andrewes and Horder on the fermentation reactions of streptococci and the very useful classification of Holman are not mentioned. Of the sporulating aerobes only B. anthracis and B. subtilis are mentioned, which would seem rather meagre in a work on General Bacteriology. The fact that, in Mallory's work on whooping cough, B. bronchisepticus was confused with B. pertussis seems to have escaped the attention of the author. There are new sections on Bacteriophage and Scarlet Fever. Discussion of the latter has been transferred from the chapter on Filtrable Viruses to that on Streptococci, but through some curious mistake reference to the work of Dochez in producing an antiscarlatinal serum is found under Epidemic Encephalitis. The author accepts much of the recent classification of bacteria proposed by the Society of American Bacteriologists. Genuses Hemophilus, Neisseria, Streptococcus, Diplococcus and Clostridium are accepted, but it seems odd that such well defined genuses as Bacterium, Corynebacterium and Bacillus are not accepted. Finally, it is odd that in none of the well known English texts is Bacillus (Corynebacterium) pyogenes mentioned, an organism that is almost as common in the suppurations of cattle and swine as is the staphylococcus in man.

Notwithstanding these specific criticisms, Jordan's Bacteriology occupies a place in the front rank of those which can be recommended to medical students and students of general bacteriology.

J. H. B.

A Textbook of Obstetrics. By THOMAS WATTS EDEN, M.D., C.M. (Edin.), F.R.C.P. (Lond.), F.R.C.S. (Edin.) and EARDLEY HOLLAND, M.D., B.S. (Lond.), F.R.C.S. (Eng.). Sixth Edition, pp. 685, with 7 Plates and 393 Illustrations in the Text. (*The Macmillan Co., New York, 1925.*)

The sixth edition of Eden's well-known textbook has been thoroughly revised by Holland, with the addition of much new material which brings it into accord with modern obstetric views and practices. In a book approximately half the size of several of the better known American texts, the reader cannot expect the detailed discussions of many of the mooted questions in Obstetrics. However, the authors are to be congratulated on their clear and unbiased—if somewhat brief—expositions of these unsolved problems. The chapters devoted to the newborn child and to the injuries, diseases and death of the foetus, are noteworthy features of the book. These subjects, which so rarely receive the attention they deserve in modern textbooks of Obstetrics, are presented here with great clarity and detail.

It is disappointing to find the subject of Prenatal Care limited to a somewhat vague summary of three pages at the end of the book. The modern obstetrician is coming more and more to feel that perhaps his most important function is to carry his patients through the period of gestation with safety and with as little discomfort as possible. Certainly this subject deserves more detailed exposition than the authors have given it.

The illustrations are worthy of favorable comment. They have been chosen wisely, reproduced with unusual clarity, and add much to the value of the book. The excellent X-ray reproductions, with line drawing explanatory keys, lead one to hope that such illustrations will play a more important röle in future texts.

J. W. H.

Modern Methods of Amputation. By THOMAS G. ORR, A.B., M.D., F.A.C.S. With one hundred and twenty-five illustrations. 117 pp. $3.50. (*The C. B. Mosby Company, St. Louis, 1926.*)

Amputation, in civil practice, is a comparatively rare operation. The total number done is not large. Therefore the operation is performed only very occasionally by the individual surgeon. The surgeon is unusual who does not want to refresh his memory on the important points of an operation which he seldom performs, whenever the occasion arises to perform it. He is also unusual if he takes the time to go through the voluminous literature to cull out the few points he is looking for. He does not want to have to look through a lot of extraneous material. What he wants is a handy reference work covering the essential points. Orr's book performs this service for the busy surgeon in the matter of amputations. It contains many useful and practical points as to the technique of amputation, choice of site, treatment of nerves, muscle, fascia, skin flaps, etc. It very happily omits the discussion of old and discarded methods. The illustrations are good, and ample.

The book also contains a chapter on the little used (in this country at least)

cinematiplastic method of amputation. There is also an excellent chapter on the very important subject of artificial limbs and artificial limb fitting.

A. R. K.

A Text-book of Pathology. By W. G. MacCallum. 3d edition. 1162 p. $10. (*W. B. Saunders Co., Philadelphia and London, 1924.*)

Medical and other text-books appear so frequently to have been compiled in a mechanical fashion rather than to have been written, in the true sense. It is as though material were gathered perfunctorily from obvious sources, condensed into the usual dogmatic formulae, and arranged in a more or less traditional manner; a few of the author's own observations and prejudices are mixed in, and the resultant tasteless gobbet is passed on bluntly and coldly to the reader. When one considers that most text-books are intended especially for young impressionable minds which are being brought into contact with the subject for the first time and therefore usually possess the curiosity and interest which a first meeting arouses, it is a matter of some wonder that so little effort is made by the authors to write of their subject in a manner designed to nourish appetizingly that nascent interest and curiosity which can be all too easily nauseated and repelled by a lifeless cataloguing of dry facts.

Si jeunesse savait . . . . ! The pity is that, as a rule, a man is not ripe to write a text-book until he has taught his subject for a long time. The young teacher is usually impressed with the beauty and fascination of his field, and is filled with a real desire to make others aware of its treasures; but after meeting the same phenomena repeatedly, and after reciting his lectures over and over to class after class, it is not uncommon that wonder becomes dimned by familiarity, and that the eagerness to awaken interest in others becomes dulled by disappointment in the average inappreciativeness. It is at this point that many teachers find themselves experienced enough to compile text-books, and impelled by sundry circumstances to do so. The uninspiring results extend across our shelves.

To a few individuals, however, there is given the power to regard every part of the accumulated experience of their maturity with the unspoiled eyes of youth, and text-books written by such persons have belonged as truly to art as to science. They have the air of having been written with pleasure, and they are provocative of interest, thought, imagination and even of original investigations. Such books are a joy to students and to specialists alike; and among that rare group one could not fail to include MacCallum's Pathology. "No doubt," writes an English reviewer, "this book has often been taken down from the laboratory shelf to verify some small point, not to be replaced immediately the information sought has been obtained; it is apt to beguile the reader, enticing him to some stimulating thought. It teaches ideas rather than facts."

The volume in hand is the finely illustrated, third, revised edition. It would be gratuitous to comment upon the soundness and accuracy of the book as a whole. The name of W. G. MacCallum on a pathological work is a more persuasive guarantee of such qualities than any recommendations of a reviewer.

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Surgical Anatomy of the Human Body. By JOHN B. DEAVER, M.D., Sc.D. LL.D., F.A.C.S. Second edition. In three volumes thoroughly revised and rearranged. Profusely illustrated with drawings from original dissections made especially for this work. Volume I. *Scalp, Cranium, Brain, Face, Mouth, Throat, Organs of Special Senses.* 1926. 4°. 551 pages. P. Blakiston's Son & Co., Philadelphia. $12.00.

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Nervous and Mental Disorders from Birth through Adolescence. By B. SACHS, M.D., and LOUIS HAUSMAN, M.D. 110 illustrations including 3 full-page plates (1 in color) 1926, 8°. 861 pages. Paul B. Hoeber, New York. $10.00.

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